TESIS DOCTORAL

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Nuevos desafíos en el análisis de la composición mineral de los alimentos

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El Prof. Dr. M. Luisa Cervera Sanz, Catedrático de Universidad, y el Prof. Dr. Miguel de la Guardia Cirugeda, Catedrático de Universidad del Departamento de Química Analítica de la Universidad de Valencia (Estudi General)

CERTIFICAN

Que D. Manuela Tamara Ruiz de Cenzano Calabuig ha realizado la presente Tesis Doctoral titulada **"Nuevos desafíos en el análisis de la composición mineral de los alimentos"** bajo su dirección en el Departamento de Química Analítica de la Universidad de Valencia, y autorizan su presentación para optar al Grado de Doctor en Química.

Y para que así conste, firman la presente en Burjassot a 26 de mayo de 2017.

Prof. Dr. M. Luisa Cervera Sanz

Prof. Dr. Miguel de la Guardia Cirugeda

A mi madre

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RESUMEN

La presente tesis doctoral integra once trabajos centrados en el desarrollo de herramientas analíticas que permitan avanzar en el conocimiento de la composición mineral de los alimentos. De acuerdo a su temática pueden clasificarse en tres bloques:

1) Perfil mineral de alimentos, que incluye una revisión de la bibliografía acerca de la composición mineral de cereales y legumbres y 3 trabajos para establecer el perfil mineral de menús infantiles de comida rápida, del pescado panga y de muestras de suplementos alimenticios.

2) Determinación de mercurio, que incluye 4 trabajos en los que se analiza la cantidad de mercurio en setas, legumbres y pescados y se desarrolla y aplica, cuando es posible, una metodología para la determinación de las especies de mercurio.

3) Determinación de arsénico, antimonio, bismuto, selenio y teluro, apartado integrado por 3 trabajos en los que se determinan estos elementos y, cuando es posible, sus especies en alimentos para bebés, yogures y productos cárnicos.

Las técnicas de análisis empleadas fueron:

- para la obtención del perfil mineral de alimentos, la espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES).
- para la determinación de mercurio, un analizador directo basado en la espectroscopia de absorción atómica tras degradación térmica de la muestra y amalgama de Hg (TDA-AAS) y la espectroscopia de fluorescencia atómica con vapor frío (CV-AFS).
- para la determinación de As, Sb, Bi, Se y Te, la espectroscopia de fluorescencia atómica previa generación de hidruros (HG-AFS).

Todos los trabajos se sustentan en la idea común del desarrollo de métodos rápidos y de coste asequible desde el punto de vista económico y medioambiental. Las metodologías han sido validadas de acuerdo con los parámetros fundamentales de la Química Analítica y su aplicabilidad, demostrada mediante el análisis de productos de mercado.

1. Composición mineral de los alimentos

La ingesta de sustancias químicas a través de la dieta constituye, para el ser humano, una de las principales vías de entrada en su organismo. Entre los muchos minerales que consumimos y acumulamos en el cuerpo solo un pequeño número de ellos se considera esencial para la vida. Una ingesta inadecuada, ya sea por exceso o por defecto, puede causar lesiones y afectar a funciones biológicas importantes, llegando incluso a provocar síntomas clínicos. Además, para ciertos elementos es fundamental, dado que su toxicidad o esencialidad depende de ello, conocer las especies que se encuentran presentes en el alimento.

Por otra parte, las sociedades modernas, caracterizadas por nuevos estilos de vida, demandan cada vez más información específica de aquello que consumimos. Nuevos hábitos alimenticios y cambios en las prácticas de elaboración y manufacturación dibujan un escenario lleno de desafíos en el análisis de la composición de alimentos. Es por tanto necesario, continuar avanzando en el desarrollo de métodos de análisis que den respuesta a esta realidad.

1.1. Seguridad alimentaria

Los legisladores europeos han creado un sistema global de evaluación de riesgos destinado a implantar índices de seguridad alimentaria fiables. En el sistema del Codex -integrado por la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO) y la Organización Mundial de la Salud (OMS)- la evaluación toxicológica para caracterizar el peligro la lleva a cabo el Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios (JECFA). Normalmente, a partir de una serie de estudios toxicológicos con animales se establece el Nivel Sin Efecto Observable (NISEO) y, dividiendo éste por un factor de seguridad, se hace una estimación de la dosis que no produciría efectos adversos en humanos y se establecen los niveles de Ingesta Mensual Tolerable Provisional (IMTP), Ingesta Semanal Tolerable Provisional (ISTP) o Ingesta Diaria Tolerable Máxima Provisional (IDTMP). El uso del término “provisional” denota que la evaluación tiene carácter transitorio debido a la escasez de datos fidedignos sobre las consecuencias de la exposición humana a niveles próximos a los considerados por el JECFA. Por ello se solicita, de manera continuada, realizar nuevos estudios tanto en el ámbito toxicológico como en el del

análisis de alimentos. En la Tabla 1 se recogen los valores de IMTP, ISTP y IDTMP establecidos por el JECFA para Al,[1] Cd,[2] Cu,[3] Fe,[4] Hg,[2] metilmercurio,[5] I,[6] Pb,[7] Sn inorgánico[8] y Zn.[3] Cabe destacar que el valor establecido como ISTP para el As inorgánico -que era de 15 µg/kg de peso corporal- fue anulado en el año 2010 y se encuentra bajo revisión. La Autoridad Europea de Seguridad Alimentaria (EFSA) ha hecho un llamamiento para poner a punto métodos analíticos validados y para obtener datos sobre las especies de As en diferentes tipos de alimentos.[9]

Tabla 1. Ingesta tolerable establecida para algunos elementos y sus especies por el JECFA

Elemento	Índice	Cantidad
Al	ISTP	2 mg/kg pc
As inorgánico	ISTP	eliminado (15 µg/kg pc)
Cd	IMTP	0,025 mg/kg pc
Cu	IDTMP	0,05-0,5 mg/kg pc
Fe	IDTMP	0,8 mg/kg pc
Hg	ISTP	0,004 mg/kg pc (excepto pescado)
metilmercurio	ISTP	1,6 µg/kg pc
I	IDTMP	0,017 mg/kg pc
Pb	ISTP	eliminado (25 µg/kg pc)
Sn inorgánico	ISTP	14 mg/kg pc
Zn	IDTMP	0,3-1 mg/kg pc

pc: peso corporal

Por otra parte, la actual legislación europea regula el contenido de determinados elementos en aquellos alimentos que son susceptibles de contener una cantidad potencialmente tóxica. El Reglamento (CE) nº 1881/2006 de la Comisión, de 19 de diciembre de 2006,[10] y sus posteriores modificaciones fijan el contenido máximo para As inorgánico,[11] Cd,[12] Hg,[11,13] Pb[14] y Sn inorgánico en diferentes productos alimenticios tal como puede verse en la Tabla 2.

Tabla 2. Contenidos máximos (mg/kg peso fresco) para metales pesados y otros elementos por grupos de alimentos Reglamento (CE) nº 1881/2006 y modificaciones					
	UE 2015/1006	UE 488/2014	CE 629/2008 UE 2015/1006	UE 2015/1005	CE 1881/2006
Alimento	**As**	**Cd**	**Hg**	**Pb**	**Sn** inorgánico
Vegetales	-	0,05/0,1/0,2/1,0	-	0,1/0,3	-
Frutas	-	0,05	-	0,1/0,2	-
Cereales	0,1/0,3	0,1/0,2	-	0,2	-
Carnes	-	0,05/0,2	-	0,1	-
Despojos	-	0,5/1,0	-	0,5	-
Productos de la pesca	-	0,5/1,0	0,5/1,0	0,5/1,0/1,5	-
Carne de pescado	-	0,05/0,1/0,2/0,3	0,5/1,0	0,3	-
Leche y sus derivados	-	-	-	0,02	-
Complementos alimenticios	-	1,0/3,0	0,1	3	-
Alimentos enlatados	-	-	-	-	50/100/200

1.2. Ingesta recomendada

Diferentes organismos, de relevancia en el ámbito de la nutrición a nivel internacional han establecido cuál es la Ingesta Diaria Recomendada (IDR) de ciertos elementos considerados esenciales. La Tabla 3 recoge los valores de IDR para Ca, Cl, Cr, Cu, F, Fe, I, K, Mb, Mg, Mn, P y sal común (Na) que figuran en el Anexo XIII del Reglamento (UE) nº 1169/2011 del Parlamento Europeo y del Consejo de 25 de octubre de 2011 relativo al etiquetado sobre propiedades nutritivas de los productos alimenticios,[15] que tiene su origen en un informe de la FAO/OMS de 1988 y los valores de IDR establecidos por la EFSA[16-28] a partir de evidencias científicas recientes.

Adicionalmente, en la Tabla 3 y para los mismos elementos, se incluyen los valores de IDR según el Institute of Medicine of the National Academies de Estados Unidos (IOM),[29] empleados también como referencia para hacer valoraciones de algunos resultados a lo largo de esta tesis.

Tabla 3. Ingesta recomendada (adulto hombre/mujer) para algunos elementos minerales			
Elemento	**(UE) No 1169/2011**	**EFSA**	**IOM**
Ca	800 mg	950 mg	1000 mg
Cl	800 mg	-	2300/2200 mg
Cr	40 µg	-	35/25 µg
Cu	1 mg	1,6/1,3	0,9 mg
F	3,5 mg	0,05 mg/kg pc	3,4 mg
Fe	14 mg	11/16 mg	8/18 mg
I	150 µg	150 µg	150 µg
K	2000 mg	3500 mg	4700 mg
Mb	50 µg	65 µg	45 µg
Mg	375 mg	350/300 mg	420/320 mg
Mn	2 mg	3 mg	2,3/1,8 mg
Na	-	-	1500 mg
P	700 mg	550 mg	700 mg
Sal común	6 g	-	-
Se	55 µg	70 µg	55 µg
Zn	10 mg	9,4-16,3/7,5-12,7	11/8 mg

pc: peso corporal

2. Determinación del perfil mineral de los alimentos

Los alimentos contienen diversos minerales en diferentes niveles de concentración. Muchos estudios se centran en el análisis de un determinado elemento que resulta de interés por sus propiedades nutritivas o, por el contrario, por su carácter dañino. Sin embargo, para caracterizar los diferentes tipos de alimentos y tener una visión global del aporte mineral que implican a la dieta resulta muy interesante poder establecer su perfil mineral. Esto es posible llevarlo a cabo en un tiempo relativamente corto mediante el uso de técnicas de análisis multielemental que permiten la cuantificación simultánea de muchos elementos como son la espectrometria de masas con plasma de acoplamiento inductivo (ICP-MS), la activación neutrónica (NAA), la fluorescencia de rayos X (FRX) o la espectroscopia de emisión óptica con plasma de acoplamiento inductivo (ICP-OES), técnica empleada en esta tesis.

2.1. El empleo de la ICP-OES en la obtención del perfil mineral de los alimentos

La ICP-OES se caracteriza por una elevada precisión, selectividad, amplio intervalo dinámico lineal y un espectro de emisión rico que permite la determinación de la mayoría de los elementos del sistema periódico. El uso de una fuente de plasma de argón a alta temperatura, muy energética y poco reactiva, permite determinar elementos que no se pueden atomizar y excitar con otras técnicas y, además, con la ventaja de que la radiación de emisión de fondo interferente es baja. Los límites de detección, aunque mayores que en la ICP-MS, son relativamente bajos, sobre todo cuando se emplea el modo de detección axial. Además, el modo operativo es sencillo y el coste relativamente pequeño en comparación con la ICP-MS.[30]

La ICP-OES es una de las técnicas preferidas para determinar el perfil mineral en alimentos puesto que implica muchas muestras en el análisis de rutina y la cuantificación de los elementos a nivel mayoritario, traza y ultratraza. En los tres trabajos llevados a cabo empleando esta técnica se planteó la determinación de 26 elementos en alimentos infantiles y de 42 elementos en pescado panga y suplementos alimenticios. Aunque los intervalos dinámicos lineales son muy grandes, se optó por preparar 3 calibrados en función del orden de magnitud esperado para los distintos elementos y así llevar a cabo una buena cuantificación. Para Ca, K, Na y Mg se calibró el intervalo 1 - 80 mg/L. Para Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Se, Sr, Ti, Tl, V y Zn se calibró el intervalo 0,05 - 5 mg/L. Y a niveles de ultratraza, para Ce, La, Nd y Pr se calibró el intervalo 0,1 – 1,0 mg/L y para Dy, Er, Eu, Gd, Ho, Lu, Nd, Sc, Sm, Tb, Tm, Y e Yb el intervalo 0,02 - 0,2 mg/L.

En el desarrollo de la metodología empleando ICP-OES para el análisis de muestras complejas -como son los alimentos- es fundamental el control del efecto de la matriz. Los restos de materia orgánica, los ácidos inorgánicos y los elementos fácilmente ionizables, como los álcalinos, afectan la formación del aerosol, el transporte y los procesos de atomización, ionización y excitación; de modo que la señal analítica es alterada y puede conducir a resultados sesgados.[31]

Para minimizar el efecto de la matriz es fundamental garantizar unas condiciones de plasma robustas, lo que se logra mediante el ajuste de los parámetros instrumentales de radiofrecuencia y flujo de gas y se evalúa mediante la relación entre las intensidades de emisión de Mg(II) / Mg(I) 280,270 nm / 285,213 nm.[32] Sin embargo, queda un efecto matriz residual que debe minimizarse por otras vías. Alternativas como la separación de la matriz, *matrix matching* o método de adición de estándar son largas y costosas. Por ello se recurrió a estrategias más sencillas consistentes en la selección del tipo de nebulizador, el grado de dilución de la muestra o el estándar interno empleado, que debe cumplir los siguientes requisitos: i) no estar presente en las muestras ii) encontrarse fuera del intervalo espectral de medida de los analitos presentes en la muestra, iii) tener una respuesta sensible y iv) ser capaz de corregir la variabilidad instrumental causada por el efecto de la matriz.[31]

Por otro lado, es también muy importante la selección de una longitud de onda adecuada para cada elemento que: i) esté lejos de interferencias espectrales causadas por otros elementos y sustancias derivadas de la alta temperatura del plasma, ii) permita hacer una buena corrección de la línea base y iii) tenga una buena sensibilidad sin llegar a saturar la señal del detector. Aunque la casa comercial recomienda una serie de longitudes de onda, las particularidades de cada muestra hacen que para ciertos elementos no sean adecuadas; por lo que se realizaron ensayos previos a 2 longitudes de onda y se eligieron las más apropiadas de acuerdo con criterios de sensibilidad, selectividad y reproducibilidad.

2.2. Tratamiento de la muestra para la obtención del perfil mineral de los alimentos

El uso convencional de la técnica de ICP-OES para el análisis de alimentos sólidos requiere una etapa de tratamiento de la muestra para la obtención de disoluciones homogéneas que puedan introducirse en el plasma. Las técnicas de extracción y digestión por vía húmeda tradicionales, que emplean grandes volúmenes de reactivos y son laboriosas, han sido sustituidas en las últimas décadas por técnicas sofisticadas como la digestión asistida por microondas, la extracción asistida por ultrasonidos y la preparación de suspensiones.

En las metodologías puestas a punto para la determinación del perfil mineral de menús infantiles, pescado y suplementos alimenticios se optó por el uso de una digestión asistida por microondas a alta presión. El uso de microondas implica un calentamiento homogéneo de la muestra por rotación dipolar y conducción iónica, lo cual lleva a una descomposición de la matriz efectiva en términos de tiempo, energía y volumen necesario de ácidos y evita problemas derivados de la formación de compuestos insolubles y de coprecipitación, frecuentes en la vía húmeda tradicional. Además, el uso de reactores cerrados disminuye el riesgo de contaminaciones o de pérdida de elementos volátiles.

Como reactivos para la digestión se empleó ácido nítrico y peróxido de hidrógeno, combinación que ha demostrado ser muy útil en la digestión de alimentos y que no genera grandes problemas en la señal analítica de técnicas de espectroscopia atómica. El ácido nítrico a alta presión y temperatura aumenta su poder oxidante y no es necesario acudir a ácidos más peligrosos como, por ejemplo, el ácido perclórico. El peróxido de hidrógeno facilita la descomposición de la materia orgánica.

En los métodos de digestión de alimentos en microondas se emplean, generalmente, 0,25 g de muestra liofilizada. Sin embargo, con el fin de poder cuantificar un mayor número de elementos, se optó por utilizar el doble, 0,5 g. Esta cantidad de muestra produciría una reacción muy exotérmica y gran liberación de gases en los reactores a alta presión, con riesgo de fugas y, para evitarlo, se hizo una pre-digestión en baño de ultrasonidos durante media hora.

En el trabajo de suplementos alimenticios se llevó a cabo también, con fines comparativos, la digestión por vía seca para así lograr un grado de preconcentración alto y tratar de cuantificar elementos a nivel de ultratraza. Mientras que en la vía húmeda 0,5 g se llevaron a un volumen final de 25 mL, en la vía seca 1 g se llevó a un volumen final de 20 mL; por lo que el factor de preconcentración fue 2,5 veces mayor. Además, con la mineralización se produce la destrucción completa de la matriz y, por tanto, se minimizan las interferencias en la etapa instrumental. Para minimizar la mayor limitación de la vía seca, la pérdida de analitos junto con compuestos volátiles, se ensayó la adición de agentes de incineración, en concreto

una mezcla de nitrato de magnesio y óxido de magnesio, acelerante del proceso de generación de cenizas.[33]

2.3. Perfil mineral de cereales y legumbres

Los cereales y legumbres son base de la alimentación en muchas partes del mundo y poseen gran valor nutricional dado su alto contenido en proteínas, carbohidratos, fibras y minerales esenciales.[34,35] Sin embargo, son susceptibles de incorporar elementos potencialmente tóxicos tales como As, Pb, Hg, Br y Cd que están presentes en aguas y suelos contaminados y son absorbidos a través de las raíces (así, por ejemplo, el arroz, considerado la mayor fuente de minerales esenciales en países en desarrollo, es también la mayor fuente de As inorgánico).[36,37] También se produce una absorción directa a través de las hojas a consecuencia de la fumigación con productos como, por ejemplo, bromuro de metilo.[38]

Se realizó una revisión de los trabajos publicados en la última década sobre el contenido mineral en cereales y legumbres. Se seleccionaron aquellas publicaciones que aportaban datos sobre el contenido mineral en muestras de consumo y se descartaron las que se centraban únicamente en el desarrollo metodológico o en el análisis de patrones o muestras certificadas. Se evaluó cuáles eran las técnicas espectroscópicas utilizadas, el tipo de tratamiento de la muestra y se recopilaron los datos de concentración de los distintos elementos.

Tal como se observa en la Figura 1, aproximadamente la mitad de los estudios utilizan técnicas multielementales, ICP-MS, ICP-OES y NAA y se centran en establecer el perfil mineral y, en el caso de ICP-MS, también en la determinación de elementos de mucho interés como el As o el Se (que están a niveles muy bajos en estas muestras y requieren mucha sensibilidad instrumental). Las técnicas de absorción y fluorescencia atómica, en sus diferentes variedades de sistema de atomización e introducción de muestra -llama (F), electrotérmica (ET), generación de hidruros (HG) y vapor frío (CV)- se emplean en alrededor de un tercio de los trabajos revisados. Por último, minoritariamente, se utilizan técnicas voltamperométricas y de espectroscopia molecular.

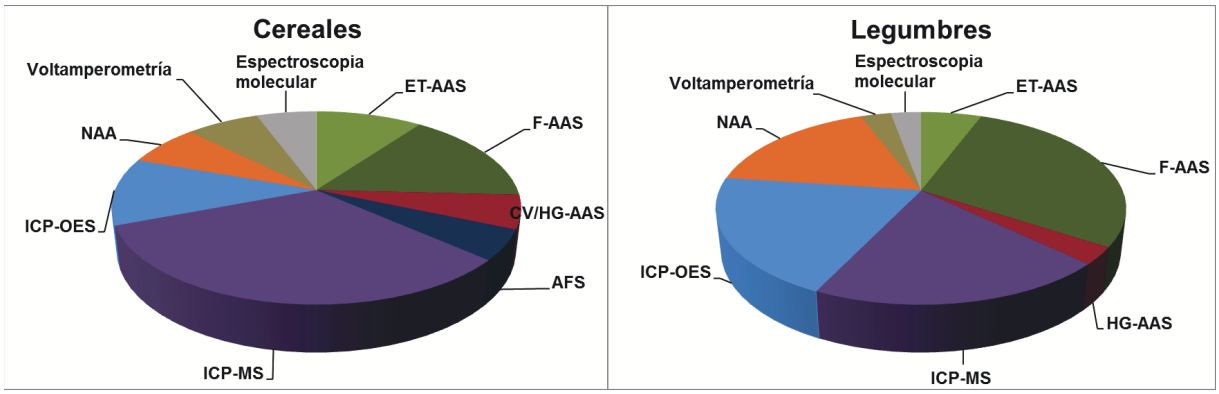

Figura 1. Técnicas empleadas para el análisis mineral de cereales y legumbres

En cuanto al tratamiento de la muestra sólida para extraer y poner en disolución los analitos (Figura 2), generalmente se usa la digestión ácida asistida por microondas cuando se va a utilizar una técnica analítica multielemental y se acude a métodos más tradicionales de digestión ácida en caliente o mineralización por vía seca cuando se emplea espectroscopia de absorción atómica.

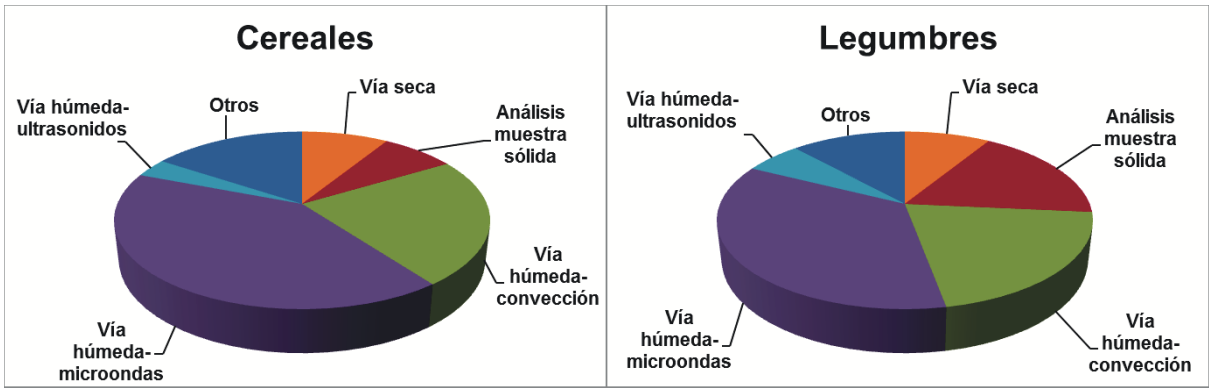

Figura 2. Tratamientos de muestra empleados para el análisis mineral de cereales y legumbres

Recopilados los datos de concentración de distintos elementos en una amplia cantidad de muestras, se estableció un perfil mineral general para cada uno de los diferentes tipos de cereales y legumbres. Al igual que otros grupos de alimentos, cereales y legumbres tienen niveles cerca del tanto por cien de K, Mg, Ca, Na y P y centenares de mg/kg de Fe, Mn y Zn. También suponen un aporte relativamente alto de Cu, Cr, Mo y Ni con niveles que van desde los centenares de µg/kg a las decenas

de mg/kg. Destaca la atención recibida por el As y el Se dada su potencial toxicidad y esencialidad, respectivamente. El As en cereales está presente en concentraciones desde centenares de µg/kg hasta los mg/kg en algunas variedades de arroz, mientras que en legumbres se encuentra en torno a las decenas de µg/kg. El Se en cereales está a niveles de µg/kg pero cabe resaltar que las legumbres son una fuente importante de este elemento, con niveles por encima de la decena de mg/kg. De otro elemento importante, dada su toxicidad, como es el Hg hay muy pocos datos en cereales y son casi inexistentes en legumbres, por lo que se decidió realizar un estudio a este respecto incluido en el apartado 2 de esta tesis.

Por otra parte, los trabajos publicados de especiación en cereales y legumbres, ciertamente escasos, emplean mayoritariamente, tras una separación cromatográfica de las especies, ICP-MS o la espectroscopia de fluorescencia atómica con generación de hidruros (HG-AFS). Algunos autores emplean métodos no cromatográficos basados en el tratamiento previo de la muestra para la extracción selectiva o en la detección selectiva de acuerdo con el comportamiento químico de las especies. El elemento más estudiado -con más de una treintena de trabajos- es el As, sobre todo en el arroz, donde la forma predominante es también la más tóxica, As inorgánico. Trabajos sobre especiación de otros elementos como Se, Hg, Te, Sb, Cr y Fe hay apenas una decena, lo cual constata la necesidad de avanzar en el desarrollo de estrategias para la especiación.

Son necesarios más estudios sobre las diferentes variedades de cereales y legumbres para descartar niveles de contaminantes nocivos, identificar áreas de cultivo adecuadas, llevar a cabo programas de enriquecimiento de cultivos y, en general, completar y actualizar las bases de datos existentes para disponer de la información necesaria que permita elaborar dietas equilibradas e incluso desarrollar programas de suplementación para elementos como el Se y el Fe, que son deficientes en las dietas de muchas poblaciones.[39,40] Además, el conocimiento de las especies de los elementos es necesario para, entre otras cosas, clarificar el mecanismo de absorción de los minerales a partir del suelo y el agua, la biodisponibilidad y los mecanismos de detoxificación en el cuerpo humano.

2.4. Perfil mineral de menús infantiles, pescado panga y suplementos alimenticios

El consumo de comida rápida ha proliferado de manera exponencial en los últimos años. Los niños son un público asiduo a menús consistentes en una hamburguesa, refresco y patatas fritas. Es comida sabrosa a precios económicos pero, por contra, muy calórica y en general poco nutritiva.[41] La mayoría de información disponible sobre este tipo de menús se refiere a macronutrientes o a unos pocos micronutrientes y, además, generalmente, se estima a partir de bases de datos de alimentos o de la información facilitada en la página web de las propias cadenas de restauración. Así pues, la falta de datos sobre minerales derivados del análisis directo de menús infantiles de comida rápida motivó la puesta a punto de un método para la determinación de 26 elementos (Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V y Zn).

Los límites de detección del método, teniendo en cuenta la cantidad de muestra liofilizada y grado de dilución empleado (0,5 g llevados a 20 mL), fueron desde 0,08 mg/kg a 1,06 mg/kg para los siguientes elementos Li, Mn, Sr, Ti, Ba, Cd, Cu, Mo, Ni, Be, Cr, Bi, Pb, Zn, Tl, V y Se, y desde 1,4 mg/kg a 26 mg/kg para Fe, Mg, As, B, Al, Na, K y Ca. La precisión del método, expresada como la desviación estándar relativa en el análisis de un triplicado, fue < 5 % para todos los elementos. La exactitud se garantizó a través del análisis de muestras certificadas de referencia de pollo (NCS ZC73016), espinacas (NIST 1570) y harina de arroz (NIST 1568a) y a través de estudios de recuperación en muestras de menús adicionadas.

Fue posible la cuantificación de 12 elementos. Las concentraciones en peso fresco, teniendo en cuenta una humedad relativa entorno al 70%, fueron del orden de ng/kg para Li, del orden de µg/kg para Ba, Cu y Mn y del orden de mg/kg para Al, Ca, Fe, K, Mg, Na, Sr y Zn.

Los resultados obtenidos se compararon con datos de la bibliografía de otros estudios de comida rápida y menús escolares, y también se calculó el aporte que supone cada elemento respecto de la ingesta diaria recomendada. Cabe destacar que elementos importantes para la dieta como Fe y K se encuentran a niveles relativamente bajos en los menús analizados en comparación con menús

convencionales, con valores entorno a los 2-4 mg/kg, lo cual supone un aporte entorno al 20 % de la IDR; mientras que el contenido en Na, alrededor de los 1000 mg/kg, es excesivo ya que representa el 70 % de la IDR, probablemente por el empleo de demasiada sal en la elaboración. No obstante, se puede garantizar que elementos tóxicos como Cd y Pb están por debajo de los límites de detección del método, que son relativamente bajos (0,04 y 0,14 mg/kg en peso fresco, respectivamente). Como limitación, la determinación de As es poco sensible y solo se puede asegurar su ausencia por debajo de su límite de detección (0,54 mg/kg en peso fresco).

El estudio del perfil mineral del panga se originó a partir del conocimiento de que es un pescado que ha entrado a formar parte de nuestras dietas recientemente y ha adquirido una gran popularidad, dado su sabor suave y precio económico, llegando incluso a sustituir el consumo de pescados blancos tradicionales como el lenguado, la merluza o el bacalao. Cuestionadas las prácticas acuícolas y la contaminación de los suelos y aguas de origen, en la literatura científica, sin embargo, hay pocos datos sobre su valor nutricional y muy pocos sobre su contenido mineral.[42] Es por esto que se puso a punto una metodología para la determinación de 42 elementos (Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tl, Tm, V, Y, Yb y Zn) y se aplicó al análisis de muestras de mercado. Adicionalmente, se determinó el Hg mediante un analizador directo basado en la espectroscopia de absorción atómica tras degradación térmica de la muestra (TDA-AAS).

Como estudios previos en el desarrollo metodológico se evaluó la posibilidad de emplear muestra fresca o muestra liofilizada. Los resultados obtenidos fueron del mismo orden de concentración, lo cual prueba que en el proceso de liofilización no se producen pérdidas ni contaminaciones. Por otra parte, la humedad relativa de las muestras de panga es elevada, del 82 %, por lo que al liofilizar se logra un factor de preconcentración en torno a 5. Sin embargo, esta preconcentración no permitió cuantificar más elementos respecto a la utilización de muestra fresca. De todos modos, finalmente, se optó por el uso de muestra liofilizada por comodidad en el manejo de muestra y proceso de conservación.

Para evaluar el efecto de la matriz se tomaron como referencia los valores obtenidos en el análisis de una muestra mediante el método de adición de patrón y se comparó con los valores obtenidos mediante calibración externa, empleando tres grados de dilución distintos de la muestra digerida, usando y sin usar un patrón interno. Se concluyó no llevar a cabo una dilución extra de la muestra, puesto que el uso de patrón interno es capaz de corregir el efecto de la matriz cualquiera que sea el grado de dilución empleado y, además, la dilución de la muestra compromete el número de elementos cuantificables e incrementa el error de cálculo.

También se hizo un estudio para comparar cómo afecta a la determinación el uso de un nebulizador ultrasónico o de un nebulizador de flujo cruzado. Se concluyó que, para las muestras de panga, se obtiene una mayor sensibilidad en el análisis con el nebulizador ultrasónico, pero el efecto de la matriz se corrige más eficazmente por el estándar interno cuando se emplea el de flujo cruzado, por lo que se optó por emplear este último.

Los límites de detección del método, teniendo en cuenta la cantidad de muestra liofilizada y grado de dilución empleado (0,5 g llevados a 20 mL), fueron desde 0,0014 mg/kg a 0,20 mg/kg para los elementos lantánidos, B, Be, Cd, Co, Cu, Li, Mn, Mo, Ni, Pb, Se, Ti y V; desde 0,24 mg/kg a 1,0 mg/kg para Al, As, Bi, Mg, Se y Tl y, desde 1,3 mg/kg a 59 mg/kg para Ca, Cr, K, Fe y Na. Las RSD para el análisis por triplicado de las muestras fueron < 30 % para los elementos traza y < 10 % para los elementos mayoritarios. Los resultados del análisis de muestras certificadas de pescado (TORT-2) fueron conformes para todos los elementos, excepto Se y Cu.

Fue posible cuantificar 14 elementos. Las concentraciones en peso fueron del orden de ng/kg para Hg, del orden de µg/kg para B, Ba, Cu, Mn, Sr y V, y del orden de mg/kg para Al, Ca, Fe, K, Mg, Na y Zn.

En comparación con datos de la bibliografía para otros pescados blancos, se observaron diferencias significativas en minerales esenciales como Fe, Sr y V, siendo estos menos cuantiosos en el panga. En general, se consideró que tiene un perfil mineral bajo con aporte de elementos como Mg, K, Fe, Zn y Cu por debajo del 2 % respecto a la IDR. Además, su alto (alrededor del 20 %) contenido en Na -que proviene del uso del aditivo E-451 (trifosfato pentasódico) para preservar el producto

congelado- contribuye al exceso de este elemento que, normalmente, está presente en nuestras dietas por un uso excesivo de sal. Elementos dañinos como Cd, Pb y Hg están por debajo de los límites recomendados por la Comunidad Europea en pescados, descartándose, así, que estas muestras se hayan visto afectadas por malas prácticas o por un ambiente que conduzca a su contaminación con metales pesados.

En el trabajo para la determinación de la composición mineral de suplementos alimenticios, elaborados a base de mezcla de cereales y azúcares, se desarrollaron y validaron dos métodos analíticos para la determinación de 42 elementos (Al, As, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Gd, Ho, K, La, Li, Lu, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Sc, Se, Sm, Sr, Tb, Ti, Tl, Tm, V, Y, Yb y Zn). Se comparó el empleo de una mineralización por vía seca con el uso de una digestión por vía húmeda seguidas de la determinación mediante ICP-OES.

En la vía seca, una vez mineralizada la muestra, las cenizas se disuelven empleando ácido diluido. Por tanto, no se requiere una dilución posterior grande de estos ácidos para que sean compatibles con el equipo de medida. Es el método de puesta en disolución más adecuado cuando se quiere determinar los elementos formadores de hidruros mediante HG-AFS. Se pretendía pues, desarrollar un método en que a partir de un único extracto obtenido por mineralización pudiera determinarse el contenido mineral mediante ICP-OES y que permitiera la medida por HG-AFS en estudios posteriores.

Dada la complejidad de la matriz, las dificultades del análisis radicaron en la extracción cuantitativa y puesta en disolución de los analitos y en la obtención de una señal analítica libre de interferencias. Por lo tanto, el estudio se centró en i) comparar los resultados obtenidos a través de la digestión asistida por microondas y la mineralización por vía seca en términos de sensibilidad, precisión y exactitud, ii) eliminar las interferencias espectrales seleccionando cuidadosamente la longitud de onda de emisión y iii) minimizar el efecto de la matriz tras ensayar diferentes grados de dilución y diferentes patrones internos (evaluando los resultados respecto a los obtenidos con un método de adición de estándar).

En el método de mineralización los límites de detección obtenidos fueron más bajos que en el de microondas ya que se empleó el doble de cantidad de muestra y un grado de dilución menor (1 g llevado a 20 mL vs 0,5 g llevados a 25 mL) y además la señal de fondo fue menor porque la destrucción de la materia orgánica es completa y se emplean ácidos diluidos. Sin embargo, a la vista de los resultados del análisis de las muestras se vio que el número de elementos cuantificables era el mismo para ambos procedimientos, por lo que se concluyó que la sensibilidad no era un factor crítico.

Los estudios de recuperación, análisis de muestras certificadas y análisis de muestras de suplementos alimenticios pusieron de manifiesto que la exactitud y precisión de la metodología que implica una digestión por vía húmeda eran en general buenas pero evidenciaron ciertas limitaciones para la vía seca. En la mineralización se demostró que era necesario el uso de un agente de incineración para evitar la volatilización de elementos generadores de hidruros como el Se o el Bi pero que sin embargo, este reactivo de baja pureza genera contaminaciones que no pudieron controlarse mediante el uso de blancos de reactivos. Lo mismo ocurrió para la determinación de Na, sesgada por el uso de material de vidrio.

Por otra parte, en cuanto a la preparación de la muestra para su análisis mediante ICP-OES, se concluyó que es necesario el empleo de un patrón interno para corregir la variabilidad instrumental causada por el efecto de la matriz, que tanto Re como Ru son adecuados para este fin y que no es necesario llevar a cabo una dilución extra de la muestra.

En relación a los niveles de concentración de elementos esenciales obtenidos en el análisis de las muestras cabe destacar que el aporte mineral de estos suplementos alimenticios es elevado, con valores de Fe, Mn y, Zn por encima de los 100 mg/kg y de Cr, Cu, Ba y Sr por encima de 1 mg/kg.

3. Determinación de mercurio

La técnica por excelencia para la determinación de Hg es la generación de vapor frío acoplada a la espectroscopia de absorción (CV-AAS) o fluorescencia atómica (CV-AFS). La presión de vapor de mercurio a temperatura ambiente permite la formación de Hg(0) gaseoso sin que sea necesario energía térmica adicional. Además, como se explicará más adelante, la AFS permite obtener muy buena sensibilidad y selectividad, superior a la absorción atómica y similar al ICP-MS y el ICP-OES, y con la ventaja de emplear una instrumentación sencilla y tener un coste relativamente bajo. Es por tanto, también, la técnica más popular para llevar a cabo estudios de especiación de mercurio.

Por otro lado, recientemente se han comercializado equipos para la determinación directa de mercurio (DMA) basados en la degradación térmica de la muestra, amalgama de mercurio y la espectroscopia de absorción atómica (TDA-AAS). Obviamente, la principal ventaja es que permite la determinación de mercurio total en muestras sólidas sin que sea necesario ningún tratamiento de las mismas y de forma rápida. También comienzan a aparecer en la literatura estudios de especiación con esta técnica aunque limitadas por uso de disoluciones compatibles con la reacción de pirolisis y con la integridad del tubo catalizador del equipo.

3.1. Mercurio. Generalidades

El mercurio es un elemento tóxico a cualquier concentración y no se le conoce esencialidad alguna. Es considerado el tóxico ambiental u ocupacional más peligroso por la gravedad de las enfermedades que causa y ha protagonizado accidentes de efectos devastadores. Es un contaminante global: una vez liberado, bien sea desde una fuente natural o antropogénica (combustión de fósiles, fertilizantes, residuos industriales…), se desplaza a través de los distintos compartimentos medioambientales y cambia de forma química, pero no se degrada. Tiene, por tanto, la característica de acumularse en las cadenas alimentarias.[43]

El grado de toxicidad del mercurio depende de su forma química. El Hg elemental es fácilmente absorbido por los pulmones mientras que el Hg^{2+} y el Hg orgánico se

pueden absorber a través del aparato digestivo. Una vez en el organismo, el mercurio, afín a grupos tiol, se une a centros catalíticos de determinadas enzimas por lo que inhibe su actividad.[44] En concreto, el metilmercurio es la especie más tóxica porque el tiempo de vida media en el organismo es muy largo, de unos 2 años, puede atravesar la barrera hematoencefálica y la barrera placentaria y puede dar lugar a efectos crónicos. La Agencia Internacional para la Investigación del Cáncer (IARC) clasifica el metilmercurio como posible carcinógeno para el ser humano (grupo 2B) y concluye que el mercurio metálico y los compuestos inorgánicos de mercurio no son clasificables en cuanto a carcinogenicidad para los seres humanos (grupo 3).[45]

3.2. El empleo de la CV-AFS y la TDA-AAS en el análisis de mercurio

La fluorescencia atómica presenta como principal ventaja una excelente selectividad, debido a que no todos los elementos presentan fluorescencia, y mayor sensibilidad que la absorción atómica, porque tiene una menor señal de fondo. La excitación empleando la longitud de onda de resonancia es selectiva para cada elemento y, por tanto, está libre de interferencias.

En este estudio se ha empleado un instrumento no dispersivo que utiliza una lámpara de cátodo hueco de descarga de excitación (BDHCL) que proporciona una emisión muy intensa en un intervalo estrecho de longitudes de onda, prácticamente a la longitud de onda del elemento considerado, por lo que no se necesita monocromador y llega mucha radiación al detector, con lo que se obtiene una mayor relación señal-ruido y, en consecuencia, muy buena sensibilidad y límite de detección.

La técnica de vapor frío (y la de generación de hidruros) permiten mejorar los límites de detección entre 10 y 100 veces respecto de otras técnicas de atomización e introducción de muestra comunes y, además, son selectivas respecto del analito, porque se produce la separación de este de la matriz. Sin embargo, el control de las condiciones experimentales es crítico y deben controlarse el estado de oxidación en que se encuentra el analito, la acidez de la muestra, la concentración del agente reductor y los caudales de transporte.[46]

En el vapor frío se prefiere como reductor $SnCl_2$ porque el $NaBH_4$ es un reductor más potente que el anterior y puede cogenerar hidruros volátiles con otros elementos de la muestra e interferir la determinación del Hg(0). Otra posible fuente de interferencias en CV-AFS es la inhibición parcial o total de la formación de Hg(0) en la fase líquida por reacciones competitivas con el agente reductor o la coprecipitación o adsorción sobre metales precipitados. Para disminuir las interferencias se acude a estrategias como diluir la muestra, aumentar la acidez o añadir agentes quelantes o complejantes.

La determinación de Hg empleando el analizador directo de mercurio se basa en la degradación térmica de la muestra, amalgama del vapor de mercurio generado en una trampa de oro y posterior determinación mediante espectroscopia de absorción atómica (TDA-AAS) tras liberación del Hg(0). Las mayores ventajas de este equipo residen en los tiempos cortos de análisis, nunca superiores a los 10 minutos, y el análisis automatizado cuando se emplea un automuestreador con capacidad para 40 muestras. Además, se pueden obtener límites de detección bajos gracias a que el equipo ofrece la posibilidad de preconcentrar el mercurio amalgamando 3 porciones sucesivas de muestra. Las condiciones experimentales críticas son el control de la contaminación, la selección de la cantidad de muestra y la selección de tiempos de secado y quemado adecuados. Así pues, la limpieza de las cubetas en las que se introduce la muestra tiene que ser exhaustiva y hay que usar blancos de reactivos a modo de control. La cantidad de muestra debe ser representativa y conducir a resultados reproducibles, pero sin ser excesiva, puesto que quemar cantidades grandes de muestra genera gran cantidad de humo que tarda en ser expulsado e incluso puede interferir la señal analítica. Si la muestra es líquida hay que alargar el tiempo de secado y si es un sólido en polvo hay que humedecerla antes, para evitar que su proyección sobre el catalizador lo inutilice.

3.3. Mercurio total en setas y legumbres

La principal vía de exposición al mercurio para el ser humano es a través de la ingesta de productos de la pesca. Sin embargo, alimentos con niveles de mercurio relativamente más bajos que el pescado, pero ampliamente consumidos, pueden

llegar a suponer un aporte de Hg a la dieta nada despreciable y por eso conviene poner a punto métodos para su control. En esta tesis se consideró interesante el desarrollo de un método para el análisis de mercurio en setas silvestres, dada la popularidad de su consumo, y en legumbres, pues, como se ha mencionado anteriormente, estas son base de la alimentación en muchas regiones del mundo y, sin embargo, apenas existen datos de su contenido en mercurio en la literatura científica.

Las setas silvestres no están sujetas a un control de seguridad alimentaria. Incluso es afición común en muchas regiones de España ir a recolectarlas al monte. Sin embargo, está demostrado que tienen facilidad para absorber y acumular metales, por lo que, de crecer en zonas contaminadas, podrían llegar a contener niveles altos de mercurio.[47]

El método elegido para el análisis de Hg en setas fue la digestión asistida por microondas con HNO_3 y H_2O_2 seguida de la determinación mediante la CV-AFS. Al adquirir un equipo de TDA-AAS, se repitieron los análisis con fines comparativos. El desarrollo metodológico para CV-AFS fue muy laborioso, dados los niveles bajos en los que se encontró el elemento, la complejidad de la matriz que interfiere en la medida y la propia naturaleza del mercurio, que hace que el método sea susceptible de pérdidas por volatilización y pueda ser objeto de contaminaciones.[48] Así pues, se extremaron las precauciones en los procedimientos de limpieza del material, en el procedimiento de digestión en microondas y, en general, en la preparación de la muestra. Para minimizar el efecto de la matriz se ensayaron varios grados de dilución y se emplearon como tratamientos de las muestra diversos agentes oxidantes y ácidos ($KBr/KBrO_3$, $KMnO_4$, $K_2Cr_2O_7$ junto con HCl y HNO_3). Finalmente fue posible la cuantificación del Hg mediante calibración externa en el intervalo 0,2 - 1,6 µg/L tras un tratamiento de la muestra con $K_2Cr_2O_7$ y ácido nítrico diluido. El límite de detección del método, teniendo en cuenta la cantidad de muestra liofilizada y grado de dilución empleado (0,5 g llevados a 50 mL), fue de 2,0 µg/kg. La precisión del método fue excelente, con una RSD de 1,2 %. La exactitud se garantizó a través de estudios de recuperación, análisis de una muestra certificada y por la concordancia con los datos obtenidos mediante TDA-AAS.

Para el análisis de una variedad silvestre de setas de consumo local, níscalos o rebollones, se obtuvieron valores de 200 - 600 µg/kg en peso seco que equivalen a 20 - 60 µg/kg en peso fresco.

Para la determinación de mercurio en legumbres se optó directamente por el uso de TDA-AAS. Aquí los niveles de Hg eran muy bajos, por lo que se trabajó con la mayor cantidad de muestra posible. Sin embargo, se observó que cantidades muy grandes de muestra conducían a resultados anómalos porque se generaban muchos humos que no daba tiempo a expulsar fuera del equipo antes del registro de la señal y ésta se veía muy interferida. Para evitar alargar el tiempo de pirólisis a alta temperatura o retrasar mucho el registro se decidió emplear una cantidad pequeña de muestra, 0,1 g, y preconcentrar el Hg de 3 submuestras en la amalgama. El límite de detección del método fue de 21 ng/kg y la precisión y exactitud adecuadas de acuerdo con los resultados del análisis por triplicado de las muestras de legumbres y del análisis de muestras certificadas, respectivamente.

Las concentraciones de Hg obtenidas en el análisis de una serie de muestras que incluían distintas variedades de lentejas, garbanzos y alubias fueron del orden de 100 - 500 ng/kg. Esto, teniendo en cuenta el consumo per capita de legumbres por ejemplo en España en 2015 que fue de 8,34 g al día supondría una ISTP para un individuo de 60 kg entre 0,1 y 0,5 ng/kg de peso corporal, muy por debajo de la ISTP de 4 µg/kg para Hg establecida por el JECFA.

3.4. Especiación no cromatográfica de mercurio

El análisis de especiación de elementos traza implica la determinación de concentraciones muy pequeñas y de especies minoritarias por lo que es muy difícil encontrar métodos que sean lo suficientemente sensibles y selectivos. Generalmente se combina una técnica de separación, en sus diferentes modalidades más comunes –cromatografía gaseosa, líquida e iónica y electroforesis capilar- con un detector muy sensible de espectroscopia de absorción, fluorescencia o emisión, o de espectrometría de masas.

En ocasiones es suficiente conocer el contenido de alguna especie o de un conjunto de estas, como por ejemplo las tóxicas, y es más factible acudir a alternativas más rápidas y sencillas, como la especiación no-cromatográfica. En concreto, estas metodologías resultan muy útiles en el análisis de alimentos como sistema de criba para el control de especies tóxicas.

Generalmente, los métodos de especiación no-cromatográfica implican estrategias basadas en la química clásica, tanto en la separación de la matriz como en el proceso de la medida. Así pues, la mayoría de los métodos para la determinación de las especies de mercurio se basan en la extracción selectiva, retención sobre un soporte sólido y en la reducción selectiva de Hg inorgánico u orgánico según el reductor empleado en el equipo de vapor frío.[49]

3.4.1. Mercurio inorgánico en setas

Los valores obtenidos de mercurio total en setas, relativamente altos, condujeron a la idea de desarrollar un método para diferenciar mercurio orgánico e inorgánico. Existen en la bibliografía varias alternativas para la determinación de mercurio orgánico en pescados. Sin embargo, no son fácilmente trasladables al análisis de setas, ya que en éstas el mercurio se encuentra mayoritariamente en forma inorgánica y en órdenes de magnitud menor, por lo que se requieren límites de detección muy bajos. Se empleó una estrategia no cromatográfica basada en la idea de que en CV-AFS el reactivo $SnCl_2$ es capaz de reducir a Hg (0) el Hg inorgánico que se encuentra libre en disolución, pero que no es capaz de reducir el Hg orgánico. Por tanto, se desarrolló un método que implica una extracción en condiciones suaves de las especies de Hg: en una alícuota se determinó directamente el Hg inorgánico y en otra alícuota se añadió un reactivo para romper el enlace de las formas orgánicas y poder, de este modo, determinar el Hg total. Se puede estimar la cantidad de Hg orgánico por su diferencia. Los puntos críticos en el desarrollo de la metodología son evitar las pérdidas por volatilización y contaminaciones, así como garantizar la no interconversión de especies. Se ensayaron distintos medios extractantes y diferentes reactivos para la ruptura del enlace orgánico. En el análisis de una serie de muestras de consumo local se obtuvieron valores de Hg inorgánico en torno a 200 - 500 ng/g en peso seco que suponen un 80 - 90% del Hg total.

3.4.2. Metilmercurio en pescados

El metilmercurio, como ya se ha indicado anteriormente, es la especie más tóxica de mercurio y se encuentra principalmente en los productos pesqueros, sobre todo en peces predadores debido a la biomagnificación a lo largo de la cadena trófica. Las autoridades sanitarias han hecho recomendaciones de limitar el consumo de pescado en aquellos grupos considerados susceptibles, como son poblaciones de pescadores o mujeres embarazadas. Sin embargo, aún están en proceso de establecer un límite máximo de metilmercurio en pescados y, de momento, los métodos de control solo contemplan el análisis de Hg total.[50]

El avance en el uso de la técnica TDA-AAS condujo a la idea de desarrollar un método para la determinación rápida de metilmercurio en pescado que pueda ser útil como método de rutina en el control de dicha especie tóxica. Para ello es necesario un pretratamiento de la muestra que, por un lado, sea capaz de extraer y separar las especies orgánicas e inorgánicas sin que estas se vean alteradas y, por otro, sea compatible con el equipo de TDA-AAS. Partiendo de diferentes propuestas de la bibliografía, se desarrolló un método rápido y sencillo, reduciendo lo máximo posible la cantidad de reactivos involucrados y el volumen de disolventes. Consiste en una hidrólisis ácida en condiciones suaves seguida de una extracción del metilmercurio en medio orgánico y retroextracción en un medio acuoso. Los ensayos previos se centraron en garantizar la estabilidad de los extractos y evitar la interconversión de especies, contaminaciones o pérdidas por volatilización. A continuación se demostró la aplicabilidad de la metodología en una serie de muestras de mercado que involucran 20 de las variedades de pescado más consumidas en el ámbito local. Las cantidades de metilmercurio halladas oscilaron entre las decenas y los centenares de ng/g, lo que representaba el 70 - 100 % del Hg total, dependiendo del tipo de pescado. Incluso para peces predadores como el atún y el emperador se detectó metilmercurio por encima del valor máximo permitido para Hg total por la legislación europea, que es de 1,0 mg/kg.

4. Determinación de arsénico, antimonio, bismuto, selenio y teluro

Arsénico, antimonio, bismuto, selenio y teluro, junto con plomo y cadmio, tienen en común la posibilidad de formar hidruros covalentes volátiles y ser determinados por HG-AFS.

Los primeros estudios empleando generación de hidruros acoplada a absorción atómica y más tarde a la fluorescencia atómica usaban equipos construidos a escala de laboratorio y se centraban en la determinación de elementos de elevado interés como el As y el Se por su potencial carácter tóxico y esencial, respectivamente. Ya a finales del siglo XX, con la comercialización de equipos de HG-AFS automatizados, estos estudios se extendieron a otros elementos generadores de hidruros.[51]

La técnica ofrece una sensibilidad muy elevada, comparable a ICP-MS, y, además, emplea volúmenes de muestra relativamente pequeños. Aunque la determinación es monoelemental, es posible llevar a cabo la determinación de varios elementos a partir de una única muestra digerida. Esto ha permitido obtener de manera adicional, con un coste relativamente pequeño, información sobre elementos como Bi, Sb o Te de los que, en el análisis de alimentos, apenas se disponía de datos.

Además, As, Se, Te y Sb se encuentran en los alimentos en diferentes formas químicas y estados de oxidación, caracterizadas por una toxicidad, biodisponibilidad y reactividad distinta. Previo un adecuado tratamiento de las muestras, la excelente sensibilidad y selectividad de HG-AFS permite obtener datos sobre especiación de estos elementos.

4.1. Arsénico, antimonio, bismuto, selenio y teluro. Generalidades

El arsénico (ver Tabla 4) es uno de los elementos más estudiados, ya que su carácter tóxico es conocido desde hace años. La química de este elemento es compleja puesto que existe en más de veinte especies químicas distintas y en diferentes estados de oxidación. La dieta es la mayor fuente de exposición de As para el ser humano. En los alimentos el As se encuentra principalmente como arsenito, arsenato, ácido monometilarsónico (MMA), ácido dimetilarsínico (DMA), arsenobetaína y arsenoazúcares.

Los estudios de As en alimentos se centran en el análisis de productos pesqueros -que suponen el grupo alimenticio de mayor aporte de As a la dieta, mayoritariamente en la forma no tóxica arsenobetaína- y en el análisis de cereales donde el contenido relativo de As total es bajo, pero, sin embargo, la proporción del As más tóxico, el inorgánico, es alta. También, es destacable, pero menos preocupante dado su consumo limitado, el contenido de As inorgánico en algas marinas. Las autoridades europeas han hecho recientemente un llamamiento a la obtención de datos de especiación de As en alimentos de diferentes tipos con el fin de poder caracterizar bien su riesgo para la salud humana.[52]

Tabla 4. Características generales del arsénico	
Origen y usos	Volcánico. Movilización a través de la minería, quema de combustibles fósiles, agricultura, ganadería, industria eléctrica y farmacéutica
Esencialidad/ toxicidad	Existen estudios que apuntan que es requerido en pequeñas concentraciones aunque su función biológica es desconocida. Muy tóxico. As inorgánico toxicidad 100 veces mayor que DMA y MMA. As^{+3} toxicidad 60 veces mayor que As^{+5}
Síntomas	Asociado a cáncer de tracto urinario, piel y pulmones. Clasificado por la IARC como cancerígeno para los humanos (Grupo I)
Exposición	A través de aguas contaminadas y alimentos como productos pesqueros, cereales y algas marinas

Junto con el arsénico a menudo coexiste el antimonio (ver Tabla 5), dada su similitud química. Sin embargo, disponemos de mucha menos información sobre este elemento. En los últimos años ha aumentado la preocupación por su existencia en el medio ambiente en concentraciones altas y ha sido declarado contaminante emergente por la OMS, por la Unión Europea y por la Agencia de Protección Ambiental de Estados Unidos.

En la dieta existe cierta preocupación sobre el Sb en productos envasados por la posible migración desde los envases de polietilentereftalato (PET). Para el resto de alimentos, de acuerdo con los pocos datos conocidos en semillas, setas, leche, pescado, zumos, vinos, productos procesados, especias y suplementos alimenticios, los niveles de Sb son bajos. Sin embargo, para poder afirmarlo con contundencia, son necesarios más datos tanto de Sb total como de sus especies y desarrollar métodos más sensibles que permitan mayor precisión en los resultados.[55]

Tabla 5. Características generales del antimonio	
Origen y usos	Natural y geológico. Movilizado por actividad minera, incineración de basuras, industria textil, automovilística y plástica, uso terapéutico antiparásitos, pesticidas, etc
Esencialidad/ toxicidad	No esencial. Toxicidad de Sb inorgánico mayor que Sb orgánico. Toxicidad de Sb^{+3} 10 veces mayor que Sb^{+5}
Síntomas	Irritación tracto respiratorio, dermatitis, queratitis, conjuntivitis, gastritis. Asociado a problemas cardiovasculares, de hígado y cáncer de pulmón. Clasificación IARC Sb_2O_3 como posible carcinógeno (Grupo 2B)
Exposición	Ocupacional, a través del aire y de la dieta, sobre todo a través de agua y comida contenida en envases PET

El bismuto (Tabla 6) en alimentos es un elemento muy poco estudiado. Los pocos datos conocidos revelan contenidos relativamente bajos en vegetales y leche. Es necesario, pues, ampliar el conocimiento a una mayor variedad de alimentos.[57]

Tabla 6. Características generales del bismuto	
Origen y usos	Industria cosmética, química y metalúrgica, y en medicamentos para combatir trastornos gastrointestinales
Esencialidad/ toxicidad	Función bilógica desconocida. Toxicidad baja puesto que se excreta rápidamente
Síntomas	Intoxicación con riesgo de nefropatía y disfunción neurológica
Exposición	A través de la dieta, se conocen algunos datos que revelan contenidos relativamente bajos en vegetales y leche

El Se (Tabla 7) es un elemento ampliamente estudiado por su importancia biológica. Se considera esencial o tóxico según la concentración a la que está presente. A partir del suelo y a través de los vegetales entra en la cadena alimentaria. Sin embargo, su presencia natural varía mucho de una regiones a otras y su disponibilidad está determinada por el tipo de alimento, especie química, pH del suelo, contenido en materia orgánica y presencia de iones complejantes de Se. Las mayores concentraciones de Se en alimentos se encuentran de forma natural en nueces de Brasil y cereales; sobre todo en trigo y sus derivados y, en menor medida, en carne, pescados, leche, huevos y soja. Además, es frecuente la suplementación de Se en las dietas, bien a nivel individual a través del consumo de complementos alimenticios, bien mediante el enriquecimiento de cultivos en terrenos deficitarios en este elemento.

Las formas químicas del Se en alimentos son muchas y las adquirimos de distinta manera: la selenocesteína, generalmente, a través del consumo de comida de origen animal y complementos alimenticios; la selenoneína a través de pescados como el atún y la caballa y la Se-metilselenocisteína y la gamma γ-glutamil-Se-metilselenocisteína a través de vegetales como el ajo, el brócoli o la cebolla.[58,59]

Tabla 7. Características generales del selenio

Origen y usos	Presencia natural en el suelo muy variable de unas regiones a otras. Empleo para el enriquecimiento de cultivos
Esencialidad/ toxicidad	Esencial o tóxico en función de su concentración. Diferentes requerimientos según edad y sexo. Funciones biológicas asociadas al papel de la selenocisteína que forma parte de 25 selenoproteínas. Efectos antivíricos, anticancerígenos en cánceres de próstata, pulmones, colorectal y vejiga, detoxificante de metales pesados Se(V), Se(VI), selenometionina y selenoglutationa son las especies más tóxicas
Síntomas	Toxicidad (seleniosis) provoca aliento de ajo, pérdida de pelo y uñas, desórdenes del sistema nervioso, mala salud dental y parálisis. La sobresuplementación de Se se asocia a diabetes tipo 2
Exposición	A través de la dieta y complementos alimenticios. Presente en nueces de Brasil, cereales, carne, pescados, leche, huevos y soja

El teluro (Tabla 8), de características químicas similares al selenio, se ha estudiado mucho menos por el hecho de no ser esencial, por lo que se disponen de pocos datos de este elemento en alimentos. Se encuentra, al igual que el Se, en nueces, cebollas, ajos y pescados y también en comidas procesadas, condimentos y productos lácteos.[60,61]

Tabla 8. Características generales del teluro	
Origen y usos	Poco abundante en la litosfera. Se obtiene como subproducto de la minería del Cu. Empleo en industria de vidrio y cerámica, refinamiento del petróleo, uso medicinal contra infecciones bacterianas, y en nuevos materiales: fluorescentes CdTe, productos fotovoltaicos.
Esencialidad/ toxicidad	Sin función biológica conocida, algunos estudios sugieren que es nutriente esencial dada su abundancia en el cuerpo humano. Tóxico a concentraciones altas. Se cree que Te(IV) es más tóxico que Te(VI)
Exposición	A través de la dieta en comidas procesadas, condimentos, cebollas, ajo, productos lácteos, nueces y pescados

4.2. El empleo de la HG-AFS en la determinación de elementos generadores de hidruros

La HG-AFS emplea un equipo análogo al de la CV-AFS, pero que incluye una llama de difusión de hidrógeno y argón como sistema de atomización y un filtro multireflectante que aísla la longitud de onda del analito y reduce emisiones debidas a la llama. La generación del hidruro se lleva a cabo empleando con $NaBH_4$ en medio ácido.

Los factores críticos en la determinación por HG-AFS son el pretratamiento de la muestra, el estado de oxidación del analito y el control de interferencias. Los elementos formadores de hidruros pueden, en muchos casos, existir en diferentes estados de oxidación que proporcionan diferentes señales analíticas; por lo que el tratamiento de la muestra debe convertirlos en un estado de oxidación común para llevar a cabo su determinación total, además de descomponer la materia orgánica y liberar el analito, superando la posible interferencia de los óxidos nitrosos.

La formación del hidruro de forma cuantitativa y rápida se produce únicamente a partir del estado de oxidación III para As, Sb y Bi y IV para Se y Te. Por tanto, para determinar contenidos totales de los elementos anteriores será necesario reducir el As(V), el Sb(V), el Se(VI) y el Te(VI). El Bi(V) es metaestable. El propio $NaBH_4$ reduce el As(V) a (III) pero no el Se(VI) y el Te(VI) que deben someterse a una etapa de pre-reducción con HCl en combinación con Br^- y en caliente. Para reducir As y Sb se usa KI y para evitar la precipitación de I_3^-, que podría interferir la generación de hidruros, se usa ácido ascórbico.

4.3. Arsénico, antimonio, bismuto, selenio y teluro en potitos

Son muchos los trabajos publicados sobre alimentos infantiles que se centran en la determinación de elementos en leche o en fórmulas infantiles. Sin embargo, actualmente, durante los primeros años de vida los potitos también juegan un papel importante en la alimentación infantil. Conocer el valor nutricional y garantizar la ausencia de sustancias tóxicas en potitos es fundamental para garantizar una buena alimentación infantil. Disponemos de bastante información sobre macronutrientes pero pocos artículos aportan datos de As o Se y prácticamente no existen datos sobre Bi, Te y Sb. En este trabajo, aprovechando la característica común de As, Bi, Te, Se y Sb de formar hidruros, el objetivo fue la determinación de los contenidos totales de estos elementos y de sus especies As(III), As(V), Sb(III), Sb(V), Bi(III), Se(IV), Se(VI), Te(IV) y Te(VI) en distintos tipos de potitos. En concreto, se consideró la determinación de las especies inorgánicas de arsénico, que son las más tóxicas, ya que muchos de los potitos están formulados a base de arroz. Se empleó un procedimiento de especiación no cromatográfica, ampliamente validado, basado en que la generación del hidruro varía en función de las condiciones de reacción. Para la especiación de Se y Te se determinó el contenido total y el de Se(IV) y Te(IV). Por su parte, Se(VI) y Te(VI), que no son formadores de hidruros, se estiman por diferencia. Para As y Sb se seleccionaron tantas condiciones experimentales como especies a determinar y se midieron patrones y muestras en distintas condiciones con el fin de obtener un sistema de ecuaciones independientes igual al número de incógnitas.

Resultan destacables los datos obtenidos de As y Se en los potitos elaborados a base de pescado, del orden del centenar de ng/g, mientras que Bi, Te y Sb están por debajo de los 5 ng/g en casi todas las muestras. Es significativamente mayor la proporción de formas tóxicas de As inorgánico en los potitos a base de pasta y verduras que en los de pescado, en los que la mayoría del As es orgánico; el Se predomina en su forma orgánica; el Bi en la inorgánica, y para Sb y Te la proporción orgánica/inorgánica varía en función del tipo de potito.

4.4. Teluro y bismuto en yogur

En la bibliografía hay numerosos estudios sobre la composición de la leche, fundamentalmente de leche materna y en menor medida de leche comercial, pero hay pocos que se centren en productos derivados en los que la composición mineral prevista de acuerdo con los ingredientes que los forman puede verse afectada por su manufacturación.[62] Encontramos en la bibliografía científica reciente una quincena de publicaciones que analizan la composición mineral de los yogures pero no se recogen datos sobre Te o Bi que, encontrándose a niveles muy bajos, requieren de métodos muy sensibles. Se consideró oportuno poner a punto un método para la cuantificación de Te y Bi en yogures y se demostró su aplicabilidad mediante el análisis de una serie de muestras de distintos tipos de yogur. El desarrollo se centró en evaluar qué alternativa de tratamiento de muestra era más adecuada, la extracción asistida por ultrasonidos a temperatura ambiente o la extracción asistida por microondas. El método de ultrasonidos, que a priori es útil para la determinación de Te y Bi en leche,[63] en el caso de yogures no lo fue, porque la materia orgánica que queda en suspensión, sobre todo en el caso de las muestras más grasas, interfiere la medida y conduce a resultados sesgados. Aplicando el método de extracción asistida por microondas a una serie de yogures de distintos tipos se obtuvieron concentraciones para Bi del orden de 1 - 10 ng/g y para Te de menos de 1 ng/g.

4.5. Formas tóxicas de arsénico en carne

El contenido de As en productos cárnicos es relativamente bajo en comparación con otros alimentos y es por eso que está menos documentado el estudio de las especies presentes. La controversia en torno al uso durante años de compuestos organoarsenicales como aditivos en piensos y fertilizantes ha impulsado el desarrollo de métodos para la determinación de algunos compuestos orgánicos del mismo.[64] Sin embargo, actualmente, el uso de ese tipo de compuestos está prohibido en la mayor parte del mundo y lo que resulta interesante es desarrollar un método rápido para diferenciar el As inorgánico, que es el más tóxico. Se conoce que las formas predominantes de As en carne son las inorgánicas As(III), As(V) y sus correspondientes metabolitos: ácido monometilarsónico (MMA) y ácido dimetilarsínico (DMA) y las orgánicas pero no tóxicas arsenobetaína y arsenoazúcares. En este trabajo, se ensayaron diferentes métodos de extracción, empleando ultrasonidos o extracción asistida por microondas, se investigaron diferentes agentes extractantes, a distintas concentraciones y con diferentes programas tiempo-temperatura. Una vez extraídas las especies de As, se aplicó un tratamiento reductor de modo que las formas de As(V) fueron reducidas a As(III). En el equipo de HG-AFS el $NaBH_4$ reacciona y forma el hidruro con el As inorgánico y las especies mono y dimetiladas pero no con las formas orgánicas de mayor metilación y de este modo pudo llevarse a cabo la determinación selectiva del total de As tóxico. La proporción de As tóxico cuantificado para una serie de muestras de productos cárnicos varió entre el 9 y el 46 % del total de As siendo este dato menor de 10 ng/g en peso fresco en el músculo y menor de 50 ng/g en peso fresco en las vísceras.

5. Conclusiones

Los métodos desarrollados constituyen aportaciones originales que han permitido poner a punto herramientas rápidas y validadas para el conocimiento de los contenidos minerales y de algunas formas químicas de los elementos de mayor interés; siendo de destacar que todos los métodos se aplicaron al análisis de muestras del mercado.

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ANEXO

1. Perfil mineral de los alimentos

1.1. Perfil mineral de cereales y legumbres

Cereals and pulses

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CHAPTER 22
Cereals and pulses

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Abstract: Available and commonly used methodologies for determining the mineral composition of cereals and pulses have been reviewed in recently published papers from 2000 to 2013. Data on the presence of major, minor, trace and ultratrace elements in barley, maize, millet, oat, rice, rye, wheat and other cereal products, together with beans, chickpeas, lentils, peas, peanuts and soybean, from different origins have been compiled to provide a picture of the state of the art in the field and the potential contribution of cereals and pulses to the daily intake of minerals.

Keywords: cereals, pulses, trace elements, arsenic speciation, selenium speciation

22.1 The importance of cereals and pulses in the daily intake of minerals

Cereals and pulses are staple foodstuffs of all the regional diets around the world; they have a key socioeconomic role in countries such as China, India, the USA and Brazil. The most important cereals include maize, rice and wheat; these three top the worldwide production rankings, just below sugar cane, with production in 2012 of 872.1, 719.7 and 670.9 million tonnes, corresponding to a trade value of US$53.6, 18.6 and 79.3 billion, respectively. With regard to pulses, soybean production in 2012 was 241.8 million tonnes, corresponding to a value of US$60.7 billion [1].

Cereals and pulses have an important nutritional value because of their high content of protein, carbohydrates, dietary fibres and minerals such as Cr, Cu, Fe, Mn, Mo, Ni, Se and Zn [2–4] and help to prevent and manage diabetes [5], cardiovascular and renal disease [6–8] and some types of cancer [9]. The most popular cereals consumed by humans are barley (*Hordeum vulgare*), maize (*Zea mays*), millet (*Pennisetum typhoides*), oat (*Avena sativa*), rice (*Oryza sativa*), rye (*Secale cereale*) and wheat (*Triticum aestivum, Triticum durum*); derived cereal products, such as pasta, breakfast cereals and bread, are commonly consumed around the world. Some of the most frequently consumed pulses include beans such as pinto beans, kidney beans and green beans (*Phaseoulus vulgaris*), mungbeans (*Phaseoulus aereus*) cowpea beans (*Vigna unguiculata*), azuki beans (*Vigna angularis*), black beans (*Vigna mungo*) and broad beans (*Vicia faba*), followed by chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), peas (*Pisum sativum*), peanuts (*Arachis hypogaea)* and soybean (*Glycine max*), and these play an important role in different cultures.

Rice is considered to be much more nutritious and a much better source of minerals than other cereals [10], especially in the developing countries due to its daily consumption [11, 12]. For example, for Chinese people, it serves as the major source of Fe, Ca and Zn [13]; it is also, together with wheat, the main source of Mn for humans [14]. However, rice is also a major source of inorganic As forms in the human diet [15]. Although most edible vegetables do not accumulate As at a high rate, rice, carrots and certain others are the exceptions [16]. Some authors have found that rice-producing areas such as Bangladesh, West Bengal, China and Taiwan typically manifest very high As contents in the soil and water, contributing to the high content of As in rice [16]. Conversely, other authors have found higher levels of As in the rice produced in the USA and Europe and have established a global 'normal' range of 0.08–0.2 μg/g in rice [17].

Regarding the mineral content of wheat, many studies have been conducted because of its numerous uses

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in derived wheat products [18]. For example, bread is a source of energy and also supplies irreplaceable nutrients to humans in many countries worldwide [13, 19–24]. Pasta obtained from wheat is a widely consumed foodstuff in Italy and is also a food item of growing popularity in many countries worldwide [22, 23, 25, 26]. Several publications have focused on how levels of minerals are affected by processing, such as the milling of wheat and derived product manufacture [27, 28]. Especially important is wheat Se input; studies have shown that the Se content of cereal crops in different countries and even different regions can vary as much as 1000 times depending on the soil [29, 30]. However, fertilization of soils and effective root uptake of Se in wheat has enabled the development of supplementation programmes to introduce this element into diets deficient in selenium [31].

The scientific literature also highlights the general importance of cereals as one of the main sources of Cr, whose required dietary intake is not usually achieved in the industrialized nations (it has even been named the 'geriatric' nutrient) [32]; a similar situation exists with Mn, Fe, Ni, Mo and Zn. Pulses are important sources of Cu, Fe, Mn, Mo, Ni and Zn, all essential elements required for good health [33, 34]. On the other hand, harmful elements such as As, Pb, Hg, Br and Cd are also present in cereals and pulses due to plant root uptake from contaminated soils and water, or by direct foliate absorption via the use of fumigants such as methyl bromide [33, 35]. Therefore it is absolutely necessary to assess the content of microelements that are considerate essential (e.g. Ca, K, P, Fe, Ni, Cu Zn, Cr, Mn, Co, Ni and Mo) and those which could be potentially toxic at high levels (e.g. Al, Ti, Ba, Br, Pb, Hg, Cd and As) [34] in both cereals and pulses due to their high consumption by humans. Data need to be collected if not available, and updated for all regions of the world. This information will be useful in setting baseline levels that may help in the preparation of balanced and therapeutic diets containing the correct amounts of trace elements [36, 37] and provides an opportunity to develop supplementation programmes for elements like Se [38, 39] and Fe [40, 41] that are deficient in the diets of many populations.

Additionally, studies on the speciation of minerals in these basic foods and the evaluation of bioavailability [13, 33, 40, 42–44] of the considered elements are of great importance in the correct evaluation of human diets in different countries.

22.2 Methods to determine the mineral composition of cereals and pulses

This chapter reviews the most relevant papers from the recent scientific literature (2000–2013) that provide data about the mineral composition of cereals and pulses. Information on sample, sample origin, analytical methodology employed and analysed elements has been summarized in five tables. Table 22.1 (rice) and Table 22.2 (wheat) have been compiled separately due to the large number of papers on these topics. In Table 22.3 we have summarized the studies on the other consumed cereals, such as barley, maize, millet, oat and rye. Table 22.4 summarizes the papers covering products derived from cereals and Table 22.5 compiles information about the mineral profile of pulses.

22.2.1 Analytical procedures

In our review of the literature, the techniques commonly used to determine the presence of mineral elements in cereals and pulses include atomic absorption spectrometry (AAS), comprising 30% of the papers reviewed, inductively coupled plasma mass spectrometry (ICP-MS) [20, 76, 140], also comprising 30% of the papers, and inductively coupled plasma optical emission spectroscopy (ICP-OES), also comprising 30%. The other techniques comprise a much smaller proportion and include methods based on atomic fluorescence spectrometry (AFS) [55, 97], voltammetry [18, 89], neutron activation analysis (NAA) [23, 29] and molecular spectrometry [117].

With regard to AAS techniques, flame AAS (FAAS) remains a popular technique for trace element determination and is usually used in combination with a preconcentration step that allows determination of measurable analyte concentrations. For example, solid-phase extraction [82, 122, 130, 158, 163], generally involving chelating resins or metal complexation followed by retention on an adsorbent support or the use of nanoparticles [50, 147] or knotted reactors [78], together with cloud point extraction [132, 134, 157] have been employed in recent years to increase the

Table 22.1 Published papers on total mineral content of rice with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Rice	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Rice	—	As	MAD	ICP-MS	[45]
Rice	China	Cd	Solid sampling	SS-ETV-AFS	[35]
Rice and rice flour	Greece, India, Thailand	As	MAD with HNO_3	ETAAS	[46]
Rice	Iran	Fe and Mn	Hot plate with HNO_3–H_2O_2	UV/Vis	[47]
Rice	Iran	Mn and Pb	Dry ashing, HNO_3	SPE-FAAS	[48]
Rice	Iran	Pb	Dry ashing, HCl	CPE-ETAAS	[49]
Rice	Iran	Pb	Heating with HNO_3	MSPE (magnetic)-FAAS	[50]
Rice	Turkey	Sb	MAD with HNO_3–H_2O_2	GFAAS	[51]
Rice	China	Se	Hot plate with HNO_3–$HClO_4$	CPE-GFAAS	[52]
Rice	China	Se	Digestive stove with HNO_3–H_2O_2	HFS-AAS	[53]
Rice	South Asia, Malaysia, Vietnam, Thailand	As	MAD with HNO_3–H_2O	ICP-MS	[54]
Rice	China	Cd	MAD with HNO_3–H_2O_2, heating with H_2O_2	Electrolytic HG-AFS	[55]
Rice	India, Pakistan, Thailand, USA, Guyana, Italy (Jamaican market)	Ag, Al, As, B, Ba, Br, Ca, Cd, Ce, Co, Cr, Cs, Cu, Eu, Fe, Hf, Hg, K, La, Mg, Mn, Mo, Na, P, Rb, S, Sb, Sc, Se, Sm, Sr, Th, Ti, U, V and Zn	Heating block with HCl–HNO_3	ICP-OES, FAAS, INAA, TXRF	[56]
Rice	China	As	Microwave with HNO_3	DRC-ICP-MS	[57]
Rice	Japan	As, Ca, Fe, K, Mg, Mn, Na, P and Zn	Microwave with HNO_3–H_2O_2	ICP-OES, ICP-MS	[58]
Rice	Taiwan, USA, India, Italy, Thailand, Canada, Sri Lanka, France, Spain, Egypt, Switzerland, Uruguay, Himalaya, Laos, Cambodia, Pakistan, China, Turkey, Madagascar, Japan	As	High-pressure asher with HNO_3	ICP-MS	[59]
Rice	Brazil	Cd	Digester block and cold finger with HNO_3–H_2O_2	ETAAS	[60]
Rice	Japan, Australia, USA, China, Thailand	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Rice	Taiwan (market)	As and Se	MAD with HNO_3–H_2O_2	DRC-ICP-MS	[62]
Rice	Spain, Japan, Brazil, India	Al, As, Ba, Bi, Ca, Cd, Ce, Cr, Co, Cu, Er, Eu, Fe, Ho, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, Pb, Pr, Se, Sm, Sr, Ti, Tl, Yb and Zn	Digestion with H_2SO_4–H_2O_2	ICP-OES	[63]

(Continued)

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice and rice semolina	Brazil (market)	As	Microwave with HNO_3–H_2O_2	DRC-ICP-MS	[64]
Rice semolina	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Rice	Italy	As	High-pressure asher with HNO_3	ICP-MS	[66]
Rice	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[67]
Rice (Se-enriched)	China	Se	Digestion block with HNO_3	ICP-MS	[68]
Rice	Brazil, Japan, Spain, Thailand	Hg	MAD with HNO_3–H_2O_2	HG-AFS	[14]
Rice and rice semolina	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Rice	—	As	Heating block with HNO_3	ICP-MS	[70]
Rice	China, Japan	Al, As, B, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, V and Zn	MAD or dry ashing	ICP-OES, ICP-MS	[71]
Rice	Italy, Spain, China, Japan, USA, Thailand, Bangladesh, Pakistan (market, internet)	As	Microwave with HNO_3–H_2O_2	ICP-MS	[72]
Rice	Turkey	As	Microwave with HNO_3–H_2O_2	SPE-HG-AAS	[73]
Rice	Japan	As	Microwave with HNO_3–$HClO_4$–HF	GFAAS	[74]
Rice	India	Se	Microwave with HNO_3–H_2O_2	DRC-ICP-MS	[75]
Rice	—	Al, Ba, Ca, Cd, Co, Cu, Fe, K, Na, Mg, Mn, Mo, Ni, P, Rb, S, Sr, V and Zn	MAD with HNO_3–H_2O_2	ICP-MS	[76]
Rice	—	Ca, Cu, K, Mg, Mn, P and Zn	—	ICP-OES	[77]
Rice	China	Cd	MAD	TS-FF-AAS	[78]
Rice	India	As	Digestion with HNO_3–H_2O_2	AHG-ICP-MS, FI-UV-HG-ICP-MS	[79]
Rice	India, Italy, UK market	As	MAD with HNO_3–H_2O_2	ICP-MS	[80]
Rice	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Rice	Turkey	Cu and Fe	Hot plate with HNO_3–$HClO_4$	SPE-FAAS	[82]
Rice	Turkey	As	Microwave with HNO_3–H_2O_2	HG-AAS	[83]
Rice	USA	As	Microwave with HNO_3	ICP-MS	[84]
Rice	China, Egypt, France, Ghana, India, Italy, Japan, Philippines, Spain, Thailand, USA	Se	Microwave with HNO_3	ICP-MS	[85]
Rice	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]

Sample	Origin	Elements	Treatment	Technique	Reference
Rice flour	Brazil	Mn	Heating with HNO_3–H_2O	FI-CPE-FAAS	[87]
Rice	China	Mn	Dry ashing	Fluorimetry	[88]
Rice	India	Cd, Cr, Cu, Fe, K, Mn, Pb and Zn	UV photolysis with HNO_3–H_2O_2	ICP-OES	[12]
Rice flour	Iran	Mn	Ultrasounds with HNO_3	AAS, ASV	[89]
Rice flour	Japan	As	MAD with HNO_3–$HClO_4$–HF	DRC-ICP-MS	[90]
Rice	Japan	Ca, Cu, Fe, K, Mg, Mn, P and Zn	MAD and heating with HNO_3–H_2O_2–HF	ICP-OES, micro-PIXE	[91]
Rice	India	As	Dry ashing	HG-AAS	[92]
Rice	West Bengal, India (market)	As	Dry ashing with ashing aid	HG-AAS	[93]
Rice	Taiwan (market)	Hg	Microwave with HNO_3	ICP-MS	[94]
Rice	USA	As	Digestion with HNO_3–H_2O_2	ICP-OES	[95]
Rice	—	As	Extraction in mild acid conditions and moderate heat	Bioluminiscence	[96]
Rice	China	Pb	MAD with HNO_3–H_2O_2	FI-HG-AFS	[97]
Rice	China	Fe and Zn	MAD with HNO_3– H_2O_2–HF	ICP-MS	[13]
Rice	Spain	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[98]
Rice	Spain, China (Libyan market)	Co, Cr, Fe, Sc and Zn	Solid sampling	INAA	[26]
Rice	Turkey	Se	MAD with HNO_3–H_2O_2	SPE-GFAAS	[99]
Rice	Turkey	Co, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	SPE-FAAS	[100]
Rice	—	As, Cd, Cr, Hg, Pb, Sb and Sn	MAD with HNO_3 and microwave-assisted evaporation	ICP-MS	[101]
Rice	Brazil	Al, Ca, Co, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Ti and Zn	—	SR-TXRF	[34]
Rice flour	Brazil	Cd, Co, Cu and Ni	Digestion bomb with HNO_3–H_2O	SPE-FAAS	[102]
Rice flour	Brazil	Fe, Mn and Zn	Focalized microwave with HNO_3–H_2O_2	FAAS	[103]
Rice flour	Brazil	Cd. Co, Ni	Digestion bomb with HNO_3–H_2O	SPE-FAAS	[104]
Rice flour	Brazil	Cu	Heating with HNO_3–H_2O	SPE-FAAS	[105]
Rice	China	Mg	Sand bath with $HClO_4$	Polarography	[106]
Rice	China (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Rice	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Rice	Korea	As, Cd, Hg and Pb	Dry ashing	ICP-OES, TDA-AAS	[108]
Rice	Uruguay	As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, Ni and Zn	MAD with HNO_3 and dry ashing with ashing aid $Mg(NO_3)_2$	ETAAS, FAAS, CV-AAS	[109]

(Continued)

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice and rice flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Rice	Brazil	Ca, Cu, Fe, K, Mg, Mn, Na, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS	[111]
Rice	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3	FAAS	[43]
Rice, flour	China	Al, Cu, Fe, Ni and V	Dry ashing	RP-HPLC-UV-Vis	[112]
Rice	Spain	As	Dry ashing	FI-HG-AAS	[113]
Rice	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Rice	Spain, India (Spanish market)	As	Ultrasound probe with HNO_3–H_2O_2. MAD with HNO_3–H_2O_2	ICP-MS	[115]
Rice and rice bean	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Rice	Thailand (market)	Ca, Fe, Mg, P and Zn	MAD with HNO_3	CFD-ICP-OES	[42]
Rice	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Rice	China	Pb	Dry ashing	UV/Vis	[117]
Rice flour	China	Cd	Hot plate with HNO_3–$HClO_4$–HCl	CV-AAS	[118]
Rice	—	As	MAD with HNO_3–H_2O_2–H_2O	Q-ICP-MS	[119]
Rice	—	Cu, Cr, Fe, La, V and Zn	Slurry PTFE	ETV-ICP-OES	[120]
Rice and rice flour	China	Cd	MAD with HNO_3–H_2O_2	UV/Vis	[121]
Rice	Turkey	Cd, Co, Cu, Fe, Ni, Pb and Zn	MAD with HNO_3–H_2O_2	SPE-FAAS	[122]
Rice	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Rice	—	As	MAD with HNO_3–H_2O_2	ICP-MS	[123]
Rice	—	As	Extraction with MeOH/H_2O 1 : 1	HG-AFS	[16]
Rice	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Rice	Taiwan (market)	Cd, Cr, Cu, Hg and Pb	Ultrasound with Triton X-100 and HNO_3 and ascorbic acid	USS-ETV-DRC-ICP-MS	[124]
Rice	China	Se	Sand bath with HNO_3–$HClO_4$	HG-AFS	[125]
Rice	China	Se	Sand bath with HNO_3–$HClO_4$	HG-AFS	[38]
Rice	Italy	As, Cd, Co, Cr, Cu, Fe, Mn, Pb, V and Zn	MAD with HNO_3– H_2O_2	ICP-OES, Q-ICP-MS, HR-ICP-MS	[10]
Rice	—	As	MAD with HNO_3	ICP-MS	[126]
Rice	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Rice	Japan	Al, Ba, Ca, Cd, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Rb, Sr and Zn	MAD with HNO_3–$HClO_4$	ICP-OES, HR-ICP-MS	[127]

Table 22.1 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Rice	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]
Rice	Vietnam	Al, As,Ca, Cd, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P and Zn	MAD with HNO_3–H_2O_2	ICP-OES, ICP-MS, FAAS	[11]
Rice	—	Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y and Yb	MW with HNO_3–H_2O_2	ICP-MS	[128]

Table 22.2 Published papers on total mineral content of wheat with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat flour	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Wheat bran	Iran	Fe	Dry ashing	Optical membrane sensor	[40]
Wheat seeds	Iran	Br, K and Na	Solid sampling	NAA	[129]
Wheat	Iran	Cd, Cu, Pb	Heating with HNO_3	SPE-FAAS	[130]
Wheat flour	Taiwan (market)	As and Se	MAD with HNO_3–H_2O_2	DRC-ICP-MS	[62]
Wheat bran, flour and semolina	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Wheat	Japan, Australia, USA, Canada	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Wheat	Italy (market)	As	Digestion block with HNO_3–H_2O_2	DCR-ICP-MS	[19]
Wheat seeds	Iran	Fe	Ultrasounds with HNO_3, hydrazine	MCPE-DSPV	[131]
Wheat flour, semolina and bran	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Wheat	Turkey	As	Microwave with HNO_3–H_2O_2	SPE-HG-AAS	[73]
Wheat flour	Brazil	Mn	Digestion bomb with HNO_3–H_2O	CPE-FAAS	[132]
Wheat	China	Ca, Cu, Fe, K, Mg, Mn, P and Zn	MAD with HNO_3	ICP-OES	[133]
Wheat	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]
Wheat	Taiwan (market)	Hg	Microwave with HNO_3	ICP-MS	[94]
Wheat flour	Iran	Mn	Ultrasounds with HNO_3	ASV	[89]
Wheat flour	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]

(Continued)

Table 22.2 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat semolina	Spain	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[98]
Wheat flour	UK	Se	MAD with HNO_3–H_2O_2	ICP-MS	[20]
Wheat	Turkey	Se	MAD with HNO_3–H_2O_2	SPE-GFAAS	[99]
Wheat	Turkey	Co, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	SPE-FAAS	[100]
Wheat	Russia	As. S, Se and Te	Heating with TMAH and HCl	Spectrophotometry UV/Vis	[135]
Wheat meal	Italy (market)	Cr, Cu, Fe, Mn, Mo, Pb, Sb, Sn and Zn	Platinum crucible with with HCl–HNO_3–H_2SO_4	SWASV, SWV, AAS	[136]
Wheat meal and wholemeal	Italy	Cr, Cu, Pb, Sb, Sn, Tl and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	ASV	[18]
Wheat flour	Libya, Italy, Tunisia (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Wheat	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Wheat flour	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Wheat flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Wheat	Serbia	As, Cd, Cu, Fe, Hg, Mn, Pb and Zn	Heating under reflux with HNO_3, $HClO_4$	FAAS, GFAAS, CV-AAS	[137]
Wheat and wheat flour	Romania	Se	Digestion bomb with HNO_3–H_2O_2	GFAAS	[30]
Wheat meal and whole meal	Italy	Cr, Cu, Pb, Sb, Sn and Tl	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV and SWV	[138]
Wheat	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Wheat meal	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Wheat and wheat flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Wheat and wheat semolina	—	Cd, Cr, Fe, Ni and Pb	MAD with HNO_3–H_2O_2	ICP-MS	[140]
Wheat	—	Cd, Cr, Fe, Ni and Pb	MAD	ICP-MS	[27]
Wheat meal and whole meal	Italy	Cr, Cu, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV	[141]
Wheat meal and wholemeal	Italy	As, Cd, Cu, Fe, Mn, Pb, Se and Zn	Block digestor with HCl	SWASV, SWCSV, SWV	[142]
Wheat	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Wheat meal and wholemeal	Italy	Cd, Cu, Pb, Sb and Zn	Block digestor with HCl	DPASV	[143]
Wheat bran, semolina flour, brown flour	—	Ag, Al, As, Au, Ba, Br, Ca, Ce, Cd, Cl, Co, Cr, Cs, Cu, Dy, Eu, Fe, Ga, Hf, Hg, I, In, Ir, K, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, Rb, Sb, Sc, Se, Sm, Sn, Sr, Ta, Tb, Te, Th, Ti, U, V, W, Yb, Zn, and Zr	Solid sampling	NAA	[144]
Wheat flour	USA, Canada, Europe	Cd, Pb, Se and Sr	MAD with HNO_3–HCl	HR-ICP-MS, MS	[28]

Table 22.2 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Wheat	—	Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Se, Sn, V and Zn	MAD with HNO_3–H_2O_2, MAD with HNO_3–$HClO_4$	Q-ICP-MS	[145]
Wheat (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]
Wheat	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Wheat, wheat flour	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampli ng	NAA	[23]

Table 22.3 Published papers on total mineral content of barley, maize, millet, oat and rye with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Barley	Turkey	Pb	Hot plate with HNO_3–H_2O_2	SPE-FAAS	[147]
Barley seeds	Iran	Fe	Ultrasounds with HNO_3, hydrazine	DSPV	[131]
Barley	Japan, Australia, USA, Canada	Sr and Pb	Digestion with HNO_3–H_2O_2	HR-ICP-MS	[61]
Barley	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Barley	—	Co, Cu, Ni and Zn	Hot plate with HNO_3–$HClO_4$	PCRBFNs, PCFFNNs	[149]
Barley	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Barley	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Barley (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]
Maize flour	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Maize flour	Brazil	Mn	Ultrasounds with HNO_3 and Triton X-100	SS-ETAAS	[14]
Maize grits and flakes	Poland	Fe	Electric plate with HNO_3–H_2O_2–H_2O	Spectrophotometric	[148]
Maize flour	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Maize bran	—	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	MAD. Clossed-vessel with HNO_3; open-vessel with HNO_3–H_2O_2–HCl	ICP-OES	[150]
Maize	—	Dy, Hf, Rb, Sc, Se	Solid sampling	PCINAA	[86]

(Continued)

Table 22.3 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Maize	Italy (market)	Se	MAD with HNO_3–H_2O_2–NaCl	HG-ICP-MS, DPASV	[81]
Maize flour	Brazil	Mn	Heating with HNO_3–H_2O	FI-CPE-FAAS	[87]
Maize flour	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]
Maize meal	Italy	Cr, Cu, Pb, Sb, Sn, Tl and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	ASV	[18]
Maize meal	Italy (market)	Cr, Cu, Fe, Mn, Mo, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV, SWV	[136]
Maize	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Maize flour	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Maize starch and flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Maize flour	Brazil	Cu	Heating with HNO_3–H_2O	SPE-FAAS	[105]
Maize flour	Czech Republic (market)	Fe, Co, Cu, Mn, Mo, Ni, P and Zn	MAD with HNO_3	ICP-MS	[152]
Maize	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Maize	Libya (market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Maize and maize flour	—	Ag, Al, Ba, Bi, Cd, Cr, Cu, Fe, Ga, In, Mg, Mn, Pb and Zn	Hot plate with HNO_3–H_2SO_4 or HNO_3–H_2O_2–H_2SO_4; or dry ashing	ICP-OES	[110]
Maize meal	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Maize	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Maize flour	China	Cd	hot plate with HNO_3–$HClO_4$–HCl	CV-AAS	[118]
Maize	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Maize	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Maize meal	Italy	Cr, Cu, Pb, Sb, Sn and Zn	Platinum crucible with HCl–HNO_3–H_2SO_4	SWASV	[141]
Maize meal	Italy	As, Cd, Cu, Fe, Mn, Pb, Se and Zn	Block digestor with HCl	SWASV, SWCSV, SWV	[142]
Maize meal	Brazil	Fe	—	Photoacoustic	[153]
Maize meal	Italy	Cd, Cu, Pb, Sb and Zn	Block digestor with HCl	DPASV	[143]

Table 22.3 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Maize	Nigeria	Fe, Zn	HNO_3–$HClO_4$	ICP-OES	[44]
Maize	Chile	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	FI-HG-AAS	[154]
Millet	China	Cd	MAD with HNO_3–H_2O_2, heating with H_2O_2	Electrolytic HG-AFS	[55]
Millet grits	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Millet	China	Mn	Dry ashing	Fluorimetry	[88]
Millet	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Millet	India	Br, Ca, Cl, Co, Cr, Fe, K, Hg, Mn, Na, P, Rb, Sc, Se, Th and Zn	Solid sampling	INAA	[107]
Millet	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Millet	—	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Sm and Zn	Solid sampling	INAA	[33]
Oat rolled and bran	Poland	Fe	Electric plate with HNO_3–H_2O_2	Spectrophotometric	[148]
Oat	Brazil	Mn	Digestion bomb with HNO_3–H_2O	CPE-FAAS	[132]
Oat	Brazil	Cu and Zn	Digestion bomb with HNO_3–H_2O_2	FS-FAAS	[134]
Oat	Russia	Se	Heating with TMAH and HCl. Oxidation with antimony (V) complex	Spectrophotometry UV/Vis	[135]
Oat flour	Brazil	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[103]
Rye flaks and flour	Spain (market)	Sb, Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[65]
Rye flakes and flour	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Rye flour	Czech Republic (market)	Fe, Co, Cu, Mn, Mo, Ni, P and Zn	MAD with HNO_3	ICP-MS	[152]
Rye	Hungary	Se	MAD with HNO_3–H_2O_2, HCl	FI-HG-GFAAS	[139]
Rye (normal and Se-enriched)	Austria	Se	—	HG-ICP-MS	[146]

Table 22.4 Published papers on total mineral content of cereal products with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Bread, pasta, biscuit and breakfast cereal	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[25]
Rice and rice crackers	Spain (market)	As	MAD with HNO_3–H_2O_2	ICP-MS	[67]
Bread	Italy (market)	As	Digestion block with HNO_3–H_2O_2	DCR-ICP-MS	[19]
Infant cereals	—	Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn	Clossed-vessel microwave with HNO_3. Open-vessel microwave with HNO_3–H_2O_2–HCl	ICP-OES	[150]
Crisped rice and puffed rice, rice crackers and noodles (breakfast cereal)	UK (market, internet)	As	Microwave with HNO_3	ICP-MS	[155]
Cuscus and pasta	Tunisia, Libya (Libyan market)	Co, Cr, Fe, Sc and Zn	Solid sampling	INAA	[26]
Bread wholemeal	—	Se	MAD with HNO_3–H_2O_2	ICP-MS	[20]
Cuscus and pasta	Libya, Italy, Tunisia (Libyan market)	Hg and Se	Solid sampling	INAA, RNAA	[29]
Biscuit and bread	Brazil	Cd and Fe	Solid sampling	SS-HR-CS-ETAAS	[41]
Maize sausage	—	As, Cd, Cr, Hg, Pb, Sb and Sn	MAD with HNO_3 and microwave-assisted evaporation	ICP-MS	[101]
Bread, biscuit and pasta	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Pasta	—	Cd, Cr, Fe, Ni and Pb	MAD with HNO_3–H_2O_2	ICP-MS	[140]
Bread and pasta	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Cereal breakfast	Spain	Cr	HNO_3–H_2SO_4–$HClO_4$	GFAAS	[32]
Bread	Turkey	Se and Zn	Solid sampling	INAA	[24]
Bread	Bulgaria	Ca, Fe, Mg and Zn	MW with HNO_3	ICP-OES	[13]
Pasta	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]

Table 22.5 Published papers on total mineral content of pulses with reference to sample treatment and analytical techniques.

Sample	Origin	Elements	Treatment	Technique	Reference
Alfalfa	—	Se	Enzymatic digestion (protease XIV)	ICP-MS	[156]
Bean (canned)	Turkey	Cd, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	CPE-FAAS	[157]
Bean (string, blank, read)	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Bean (broad)	Iran	Cd, Co, Cr, Cu, Fe, Pb and Zn	Hot plate and dry ashing with HNO_3–H_2O_2–HCl–$HClO_4$	SPE-FAAS	[158]

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Bean (cowpea)	Brazil	Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Pb, Sb, Se, Sr, V and Zn,	MAD with HNO_3–H_2O_2	ICP-OES, ICP-MS, DRC-ICP-MS	[159]
Bean (kidney)	India (market)	As	Dry ashing with ashing aid	HG-AAS	[93]
Bean (black, green)	—	K	Solid sampling	NAA	[160]
Bean	Brazil (market)	Al, Ca, Co, Cr, Cu, Fe, K, Mn, Mo, Ni, P, Pb, Ti and Zn	—	SR-TXRF	[34]
Bean	Brazil	Br, Ca, Co, Cs, Fe, K, Rb and Zn	Solid sampling	INAA	[41]
Bean (mung)	China	Mg	Sand bath with $HClO_4$	Polarography	[106]
Bean (kidney)	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Bean (green, mung)	India	Cr, Mo and W	—	ICP-OES	[161]
Bean (black, red, kidney, etc.)	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Bean (cowpea, mung)	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Bean (green, mung)	India	Cr, Mo and W	—	ICP-OES	[161]
Bean (green)	Italy	Mn	Heating with HCl	dASCP	[162]
Bean (broad)	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Bean	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Bean (mung, cowpea)	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Bean (mung)	Thailand (market)	Ca, Mg, P, Fe and Zn	MAD with HNO_3	CFD-ICP-OES	[42]
Bean	—	Ba, Ca, Mg, Mn, Sr, Fe, Co, Ni and Se	HNO_3	ICP-OES	[164]
Bean	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Bean	Chile	As	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	FI-HG-AAS	[154]
Bean (haricot, kidney)	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Bean	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Bean (red, mung, horse gram)	India	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Se, Sm and Zn	Solid sampling	INAA	[33]
Bean (black, red, mung)	Japan, India	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]
Bean (green)	Canada	B, Ca, Cl, Co, Cu, I, K, Mg, Mn, Na, Rb, S, Ti and V	Solid sampling	NAA	[23]

(Continued)

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Chickpea	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Chickpea	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Chickpea	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Chickpea	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Chickpea	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Chickpea	India	Al, Br, Ca, Cl, Co, Fe, K, La, Mg, Mn, Na, Rb, Se, Sm and Zn	Solid sampling	INAA	[33]
Chickpea	India	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]
Lentil	—	Se	Enzymatic digestion (protease XIV)	ICP-MS	[156]
Lentil	Iran	Fe	Ultrasound with HNO_3, hydrazine	MCPE-DSPV	[131]
Lentil	Spain (market)	As, Bi, Sb, Se and Te	Dry ashing with ashing aid $Mg(NO_3)_2$–MgO	HG-AFS	[69]
Lentil	—	Se	MAD with HNO_3–H_2O_2	ICP-MS	[167]
Lentil	Turkey	Fe, Mn and Zn	MAD with HNO_3–H_2O_2–HCl	FAAS	[37]
Lentil	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Lentil	Spain	Fe, Mn and Zn	MAD with HNO_3–H_2O_2	FAAS	[114]
Lentil	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Lentil	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Lentil	Pakistan	As, Br, Hg, Sb and Se	Solid sampling	INAA	[36]
Lentil	Czech Republic (market)	Co, Cu, Fe, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS, ICP-OES	[168]
Pea (canned)	Turkey	Cd, Cu, Fe and Pb	MAD with HNO_3–H_2O_2	CPE-FAAS	[157]
Pea	Iran	Cd,Cr, Co, Cu, Pb and Zn	Hot plate and dry ashing with HNO_3–H_2O_2, HCl–$HClO_4$	SPE-FAAS	[158]
Pea (canned)	Brazil	Cu	HNO_3–$HClO_4$	FAAS	[21]
Pea	Czech Republic	Co, Cu, Fe, P, Mn, Mo, Ni and Zn	MAD with HNO_3	ICP-MS	[169]
Pea	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Pea	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Pea	Czech Republic (market)	Co, Cu, Fe, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2	ICP-MS, ICP-OES	[168]
Pea	Japan	Al, Ba, Br, Ca, Ce, Cl, Co, Cs, Dy, Eu, Fe, K, Lu, Mg, Mn, Na, Rb, Sb, Sc, Sm, Sr and Zn	Solid sampling	NAA, PAA	[166]

Table 22.5 (Continued)

Sample	Origin	Elements	Treatment	Technique	Reference
Peanut seeds	China, Japan	B, Ba, Ca, Cd, Co, Cu, Fe, Ga, K, Mg, Mn, Mo, Na, Ni, P, Rb, Sr, and Zn	MAD with HNO_3–H_2O_2–HF	ICP-MS	[170]
Peanut	Australia	B, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Se and Zn	MAD with HNO_3–H_2O_2–H_2O	ICP-OES, ICP-MS, DRC-ICP-MS	[171]
Peanut	Greece	Al, Ba, Cd, Cr, Cu, Fe, Mg, Mn, Pb and Zn	MAD with HNO_3–H_2SO_4–H_2O_2	ICP-OES	[172]
Peanut	Ghana	Ba, Br, ca, Cl, Co, Cu, Dy, K, Mg, Mn, Na, Rb, S, Sr, Ti, Th, U, V and Zn	Solid sampling	INAA	[151]
Peanut	Spain	Cd	Continuous ultrasound with HNO_3–H_2O	FI-SPE-FAAS	[163]
Peanut	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]
Peanut	Chile	Cu, Fe and Zn	Digestion with HNO_3–H_2SO_4–$HClO_4$	AAS	[22]
Peanut	Spain (market)	Al, Cd, Cr, Cu, Fe, Ni and Pb	Digestion block with HNO_3	ETAAS	[165]
Peanut	Spain (market)	Cu and Fe	Digestion with H_2SO_4–H_2O_2	FAAS	[173]
Peanut	Italy (market)	Ca, Cu, Fe, Mg and Zn	Dry ashing, HNO_3	FAAS	[174]
Bean	Brazil	Se	MAD with HNO_3–H_2O_2, heating with HCl	CF-HG-ETAAS	[175]
Soybean meal	Iran	Co, Cu, Ni and Zn	Hot plate with HNO_3–$HClO_4$	PCRBFNs, PCFFNNs	[149]
Soybean	Burkina Faso, France, China, Senegal, Madagascar	Fe and Zn	Dry ashing and hot plate with HNO_3, HCl	FAAS	[43]
Soybean	Thailand	Se	Heating block with HNO_3–$HClO_4$–HCl	Fluorimetry	[116]

sensitivity of FAAS for trace element determination of cereals and pulses.

Despite the general trend observed worldwide of the declining use of single-element techniques in favour of multi-element analysis of foods, in the case of cereals the growing concern about As in rice and Se supplementation in wheat has led to numerous studies focused only on one of these elements. In fact, the number of reviewed papers is similar for both multi-element and single-element determinations. Studies aiming to establish the mineral profile of cereals or pulses obviously use multi-element determination techniques such as ICP-OES and ICP-MS, although several studies employed NAA and even voltammetry techniques. For As and Se determinations, the technique of choice should be ICP-MS due to its excellent sensitivity, followed by electrothermal AAS (ET-AAS), hydride generation AAS (HG-AAS) or AFS. Other elements of interest such as Cu, Cd, Fe, Mn and Pb are determined by using the aforementioned AAS techniques and also by ultraviolet/visible (UV/Vis) spectrophotometry.

22.2.2 Sample digestion

Most procedures employed for mineral determination in cereals and pulses require previous sample decomposition, with the exception of NAA methods which are

suitable to be applied directly to the dry sample. In the reviewed literature, wet digestions (85%) are clearly preferred over dry ashing (15%) [37, 110, 114]. For wet sample treatment, the use of microwave-assisted digestion (MAD) is twice as common as the use of convective heating via hot plates, digestion blocks, bomb digestors, high-pressure ashers and sand baths, and the recent use of ultrasound-assisted digestion. MAD is being increasingly used since this method facilitates rapid dissolution of the sample matrix, requires low amounts of acids and oxidizing reagents and causes minimal contamination of the sample prior to element analysis, being generally used in closed reactors under pressure. Usually MAD precedes analysis by ICP-OES and ICP-MS, whereas dry ashing and wet digestion with convective heating precede AAS. With regard to the use of acids in wet digestion of both cereals and pulses, procedures use a mixture of HNO_3 and H_2O_2 extensively (around 40% of methods), followed by the use of only HNO_3 (15%). Other alternatives involve the single or combined use of HCl, H_2SO_4, $HClO_4$ and HF.

22.3 Mineral composition of cereals and pulses

Figure 22.1 shows the concentration ranges of trace elements determined in rice from published studies. As can be seen, K, Mg and P are the elements present in highest concentrations in rice samples at thousands of micrograms per gram, followed by Ca, Fe, Mn, Na, Rb and Zn and finally by Cu, all at microgram per gram levels. Ba, Cr, As, Mo and Ni have concentrations up to 100 ng/g, whereas Cd, Se, Pb, Sc and Co are present in samples with some fluctuations and Hg is under 100 ng/g.

Figure 22.2 shows the concentration ranges of trace elements determined in wheat from recently published studies. As can be seen, K, Mg and Ca can be found at the highest concentrations (μg/g) followed by Fe, Mn, Na, Rb and Zn and finally by Cs and Al. Mo and Ni are clearly in the range 100–1000 ng/g, while Pb and Se exhibit fluctuations at the aforementioned levels. As, Cd, Co, Cr and Hg are present at the lowest concentrations. In comparison with rice, wheat exhibits a significant positive difference in the content of Cs and probably a negative difference in the content of Cu. Wheat has a slightly higher concentration of Ca but lower concentrations of As and Cr compared with rice, these differences being about one order of magnitude.

Figure 22.3 shows average data from studies of different cereals. Sodium in barley, maize and millet is the element with the highest concentration (at percentage level), followed by K, Mg and Ca. Fe, Mn and Zn are in the range 10–100 μg/g, whereas Cu and Cr are below 10 μg/g. As, Se, Pb, Hg, Cd, Ni and Mo are present at the nanogram per gram level but it is difficult to draw general conclusions because of fluctuations in their concentrations as a function of type of sample and sample origin. The high amount of Pb in barley and As in maize should be emphasized. It can also be seen that cereals exhibit higher levels of Na and Cr in comparison with rice and wheat.

Concentrations of elements in pulses are summarized in Figure 22.4. The six types of pulses have similar mineral profiles. K is the element with the highest concentration (at percentage level), while P, Mg and Ca are present in the upper thousands of micrograms per gram. Fe followed by Zn, Mn and Na are in the range 100–1000 μg/g range and Cu, Mo and Ni are also present at low microgram per gram levels. At the nanogram per gram level, Se, Cd, Co, Pb, As, Cr and Hg are reported for many pulses. The high content of Se in beans, Mo in lentils and Mn and Ni in peanuts should be noted. In comparison with cereals, pulses have high levels of K, Ca, Fe, Cu, Mo and Ni and low levels of Na and Cr.

22.4 Speciation of mineral compounds in cereals and pulses

Traditionally, speciation studies have focused on foods of marine origin and meat, being less concerned with fruits, legumes and cereals. However, recent studies have stressed the importance of speciation in foods like rice and wheat that are consumed worldwide and which can extract relatively high amounts of toxic elements such as As, Hg, Sb and Te from contaminated soils and water. Moreover, the development of very sensitive analytical tools for the determination of these elements based on HG-AFS and ICP-MS, with detection in liquid chromatography, has increased the number of studies in this field. As can be seen in Tables 22.6 and 22.7, studies of As, Se Sb, Te and Hg have been published in the last few years.

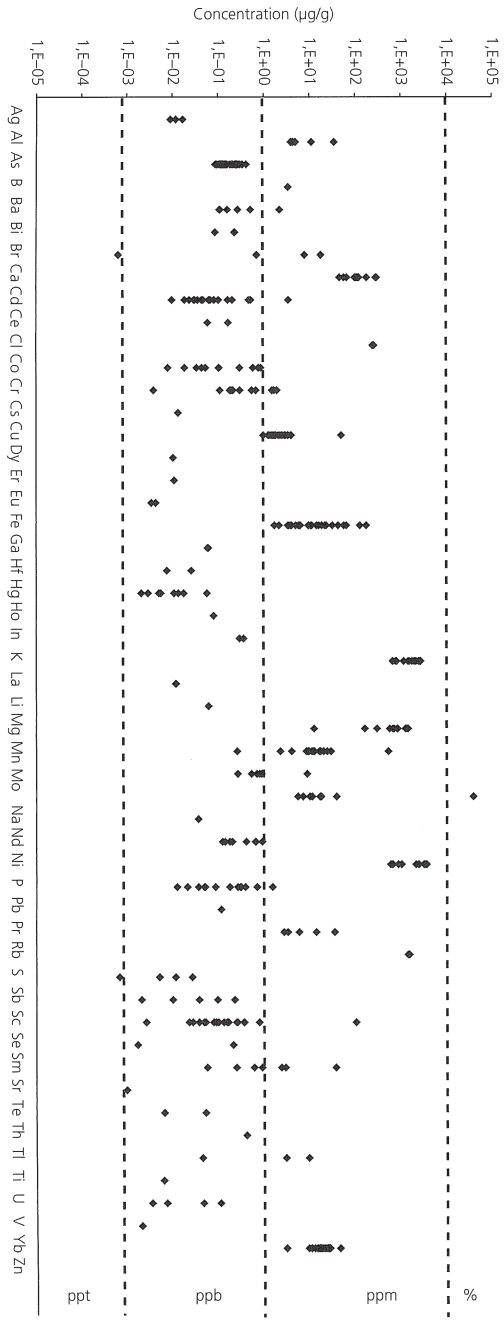


Figure 22.1 Concentration ranges of elements determined in rice from published studies.

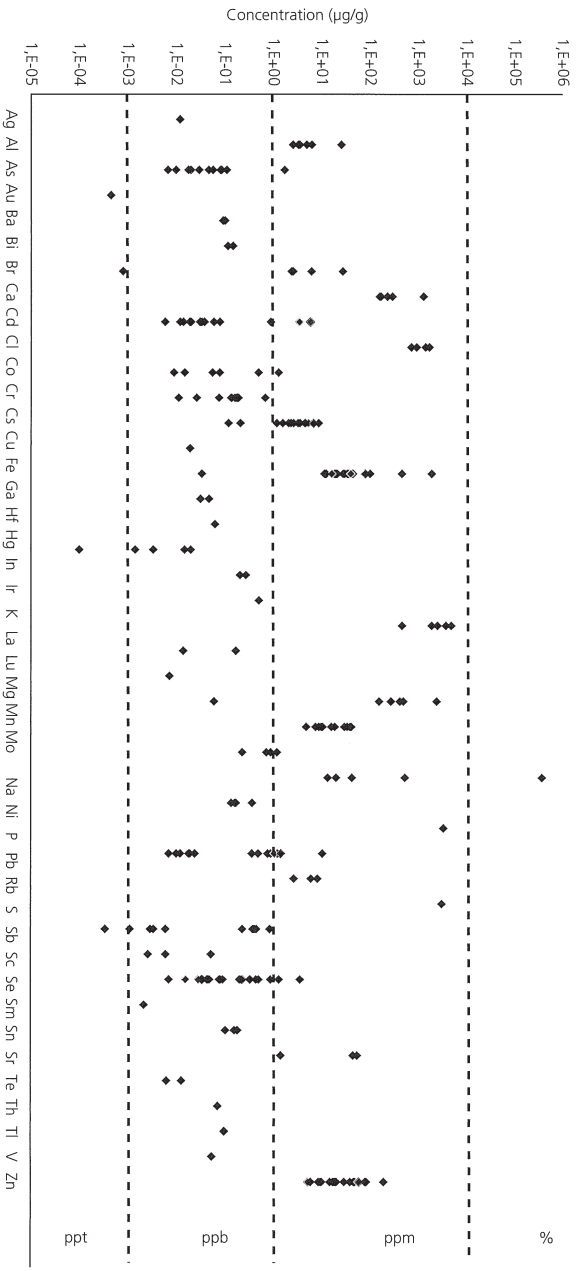


Figure 22.2 Concentration ranges of elements determined in wheat from published studies.

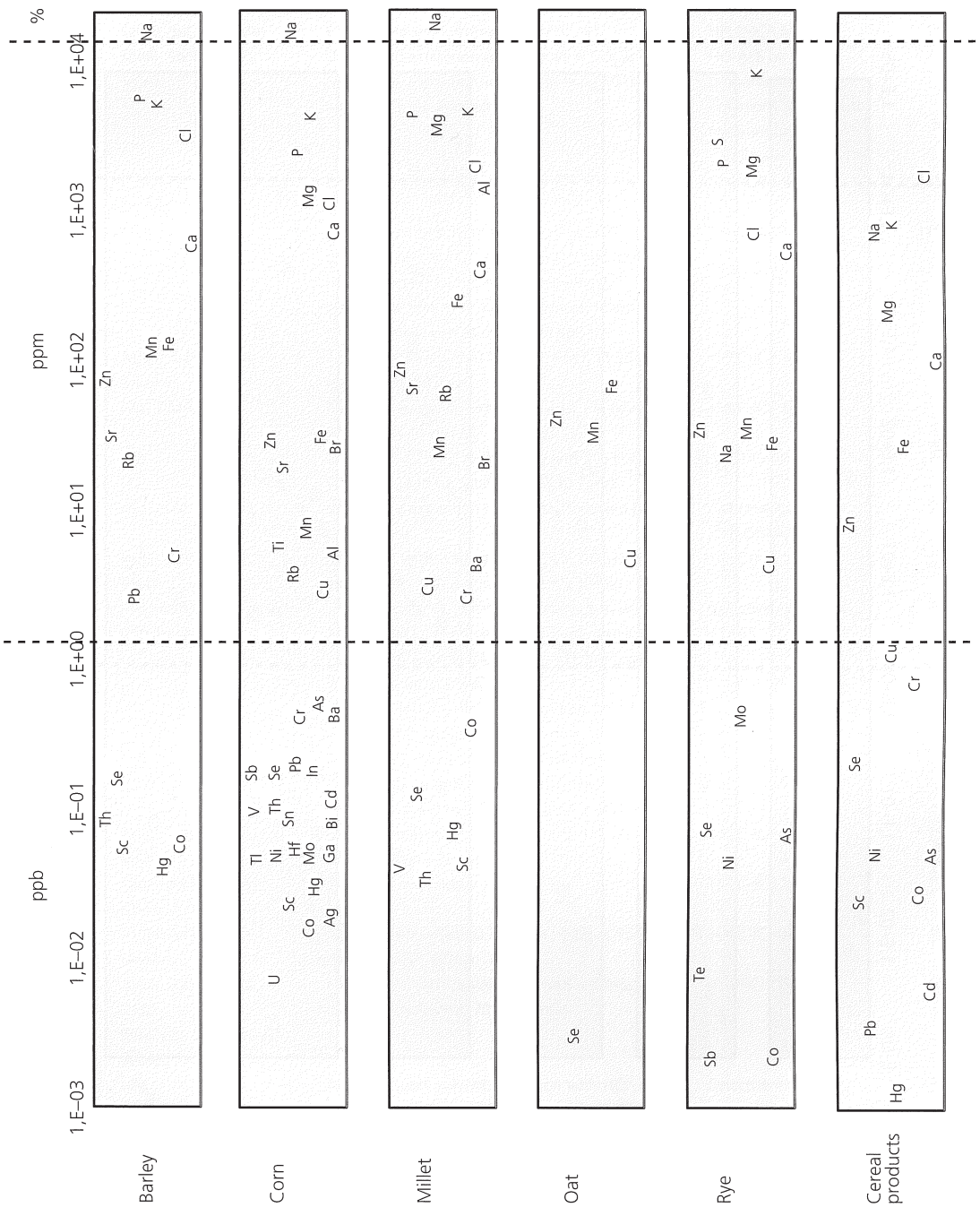


Figure 22.3 Concentration ranges of elements determined in other cereals and cereal products (bread, pasta, breakfast cereals) from published studies.

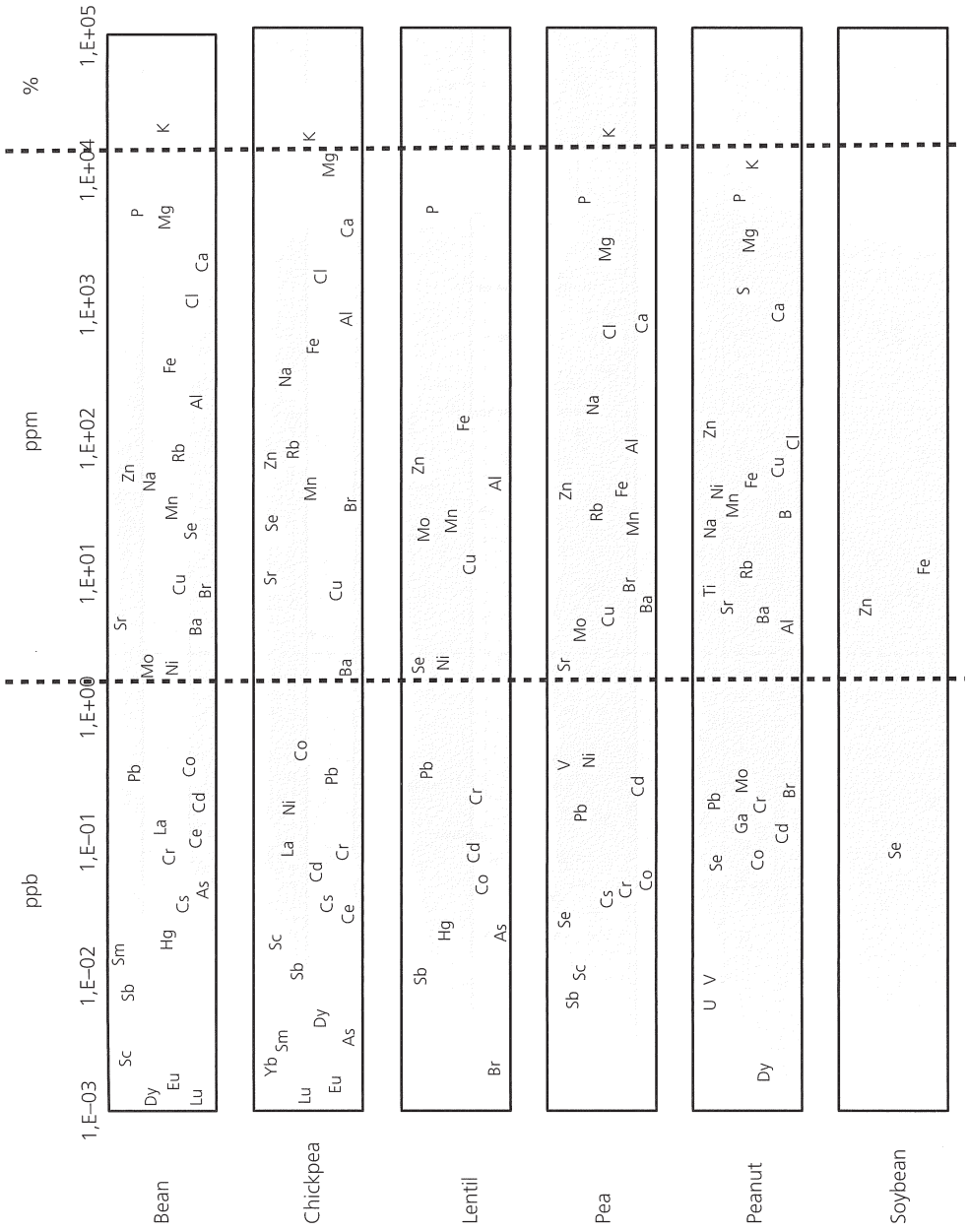


Figure 22.4 Concentration ranges of elements determined in pulses from published studies.

Table 22.6 Published papers on speciation of minerals in cereals with reference to sample treatment and analytical techniques.

Sample	Species	Treatment	Technique	Reference
Barley	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Maize flour	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Maize flour	P, Mn, Fe, Co, Ni, Cu, Zn, Mo	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[152]
Maize	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[154]
Rice and bulgur	As(III), As(V), DMA, MMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C water bath, 90 min	HPLC-ICP-MS	[45]
Rice	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Rice and rice cracker	i-As	Extraction with 0.1 mol/L HNO_3 and 3% H_2O_2 at 90 °C, 1 h, and oxidation all i-As to As(V)	SPE-HG-AAS	[177]
Rice	i-Se, o-Se	Ultrasound extraction with water followed by cyclohexane	Selective extraction, CPE-GFAAS	[52]
Rice	As(III), As(V), DMA, MMA	Extraction with water at 90 °C, 4 h, followed by enzymolysis with α-amylase 37 °C, 30 min	HPLC-ICP-MS	[54]
Rice, rice flour	i-As, As(III), As(V) by difference	*Total inorganic As*: ultrasound extraction with 1 mol/L HNO_3, 15 min and centrifuge, extraction with 0.1 % (w/v) EDTA vortex and centrifuge. Supernatants to ETAAS *As(III)*: microwave-assisted extraction 1 mol/L HNO_3, 85 °C, liquid–liquid extraction with EDTA-APDC-MIBK vortex and centrifuge. Organic phase to ETAAS for As(III), and determine As(V) by difference from T-iAs	Selective extraction, ETAAS	[46]
Rice	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 1% v/v HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[57]
Rice	As(III), As(V), DMA, MMA	Extraction with water at 90 °C, 3 h	HPLC-ICP-MS	[58]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C heating block, 90 min	HPLC-ICP-MS	[59]
Rice	As(III), As(V), MMA, DMA $(Se\text{-}Cys)_2$, SeMeSeCys, SeMet, Se(IV), Se(VI)	Enzymolysis with protease XIV and α-amylase microwave-assisted, 70 °C, 30 min	IC-DRC-ICP-MS	[62]
Rice	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice) and dilution	Selective extraction, ICP-MS	[67]
Rice	Se(IV), Se(VI), $SeCys_2$, SeMet	Enzymolysis with protease and lipase at 37 °C, 16 h	CE-ICP-MS	[68]

(Continued)

Table 22.6 (Continued)

Sample	Species	Treatment	Technique	Reference
Rice and rice semolina	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 2% v/v HNO_3 at 95 °C water bath, 90 min	HPLC-ICP-MS	[64]
Rice crackers	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice) and dilution	Selective extraction, ICP-MS	[67]
Rice	As(III), As(V), MMA, DMA	Acid extraction with 0.28 mol/L HNO_3 at 95 °C heating block, 90 min	HPLC-ICP-MS	[70]
Rice	Se(VI), SeMet, MeSeCys	Ultrasound-assisted enzymolysis with α-amylase 60 s and protease 180 s	HPLC-ICP-DRC-MS	[75]
Rice	As(III), As(V), DMA, MMA	Acid extraction with 0.15 mol/L HNO_3 at 100 °C heating block, 2 h	HPLC-ICP-MS	[72]
Rice	As(III), As(V), DMA	Water extraction at 90 °C heating block, 3 h	HPLC-ICP-MS	[74]
Rice	As(III), As(V), MMA, DMA, AsB	Extraction with methanol/water (1 : 1), 10 h at 55 °C	LC-UV-HG-ICP-MS	[79]
Rice	Se(IV), Se(VI), $SeCys_2$, SeOMet, SeMet	Ultrasound -assisted enzymolysis with α-amylase at 37 °C, 30 min and protease XIV at 45 °C, 2 h	RP-HPLC-ICP-MS, SAX-HPLC-ICP-MS	[39]
Rice	i-As = As(III + V), o-As = MMA + DMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[80]
Rice	As(III), As(V), DMA, MMA Se(IV), Se(VI), SeMet	Microwave-assisted enzymolysis with protease XIV and α-amylase 37 °C, 30 min	IC-ICP-MS	[178]
Rice	i-As, DMA, MMA	Microwave-assisted extraction with 0.5 mol/L trifluoroacetic acid at 80 °C, 10 min	HPLC-ICP-MS	[84]
Rice	Se(VI), SeMet, SeCys, MeSeCys		Micro-XANES	[85]
Rice (crackers, crisped, noodles, puffed)	i-As, o-As	Microwave-assisted extraction with 1% v/v HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[155]
Rice flour	As(III), As(V), MMA, DMA	Microwave-assisted extraction with water at 80 °C, 30 min	HPLC-ICP-MS	[90]
Rice	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[92]
Rice	As(III), As(V), DMA	Ultrasound-assisted extraction with 2 mol/L trifluoroacetic acid at 75 °C, 6 h	HPLC-ICP-DRC-MS	[95]
Rice	Hg(II), MeHg, EtHg	Microwave-assisted extraction with 0.5% (v/v) 2-mercaptoethanol in 5% methanol at 60 °C, 3 min	LC-VG-ICP-MS	[94]
Rice	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[93]

Sample	Species	Treatment	Technique	Reference
Rice	As(III), As(V), MMA, DMA	Ultrasound extraction with 1 mol/L H_3PO_4 + 0.1% (w/v) Triton XT-114 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[98]
Rice and rice cracker	As(III)	(1) Grinding rice to powder, (2) mixing with an aqueous solution containing pancreatic enzymes, (3) mechanical shearing, (4) extraction in mild acid conditions and moderate heat, and (5) centrifugation and pH neutralization	Bioluminescence	[96]
Rice	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[113]
Rice	As(III), As(V), MMA, DMA	Ultrasound-assisted enzymolysis with protease XIV and α-amylase, 3 min	LC-ICP-MS	[115]
Rice and rice cereals	As(III), As(V), MMA, DMA, AsB	Ultrasound extraction (three times) with 25, 25 and 20 mL methanol/water (1 : 1) for 1 h, 30 min, 30 min and centrifuge. Reduction volume to 5 mL	HPLC-ICP-MS	[179]
Rice	As(III), As(V), MMA, DMA	Ultrasound extraction with methanol/water (1 : 1) at 50 °C, 2 h, centrifuge. Repeat extraction four times. Concentrate the supernatants to 0.5 mL at 50 °C, 6 h, and dilute to 15 mL	HPLC-Q-ICP-MS	[119]
Rice	As(III), As(V), MMA, DMA, AsB	Ultrasound extraction with methanol/water (1 : 1) at 55 °C, 10 h, centrifuge. Repeat extraction twice. Combined extracts evaporated to dryness and diluted	HPLC-ICP-MS	[16]
Rice and rice cereals	As(III), As(V), MMA, DMA	Ultrasound extraction with methanol/water (1 : 1), 30 min, centrifuge. Repeat extraction twice. Combined extracts evaporated and diluted	HPLC-ICP-MS	[180]
Rice	As(III), As(V), MMA, DMA	Enzymolysis with α-amylase 60 °C, 24 h	IC-ICP-MS	[123]
Rice	As(III) + As(V), MMA, DMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	IC-ICP-MS	[126]
Rye flakes and flour	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Rye flour	Fe, Co, Cu, Mn, Mo, Ni, P, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[152]
Rye	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Wheat flour	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Wheat	Se(VI), SeMet	Enzymolysis with Tris-HCl buffer with protease and lipase, incubation at 37 °C, 18 h and centrifuge. Repeat the procedure with fresh buffered enzyme solution	HPLC-ICP-MS	[20]

(Continued)

Table 22.6 (Continued)

Sample	Species	Treatment	Technique	Reference
Wheat bran, flour, semolina	Sb(III), Sb(V), Te(IV), Te(VI)	Ultrasound extraction with 1 mol/L H_2SO_4 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[65]
Wheat flour	As(III), As(V), MMA, DMA $(SeCys)_2$, SeMeSeCys, SeMet, Se(IV), Se(VI)	Enzymolysis with protease XIV and α-amylase microwave-assisted 70 °C, 30 min	IC-DRC-ICP-MS	[62]
Wheat	Hg(II), MeHg, EtHg	Microwave-assisted extraction with 0.5% v/v 2-mercaptoethanol in 5% methanol at 60 °C, 3 min	LC-VG-ICP-MS	[94]
Wheat	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 0.16 mol/L HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[19]
Wheat semolina	As(III), As(V), MMA, DMA	Ultrasound extraction with 1 mol/L H_3PO_4 + 0.1% (w/v) Triton XT-114 and centrifuge, extraction with 0.1% (w/v) EDTA and centrifuge. Mix extracts	HG-AFS by proportional equations at different measurement conditions	[98]
Wheat	Se(VI), SeCys, SeMet	Enzymolysis with protease at 37 °C, 4 h	HPLC-ICP-MS	[146]
Biscuit, bread, breakfast cereal, pasta, maize snack	i-As, DMA, MMA	Microwave-assisted extraction with 1% (v/v) HNO_3 + 1% (v/v) H_2O_2	HPLC-ICP-MS	[25]
Bread and pasta	As(III), As(V), DMA, MMA	Microwave-assisted extraction with 0.16 mol/L HNO_3 at 95 °C, 30 min	HPLC-ICP-MS	[19]

Table 22.7 Published papers on speciation of minerals in pulses with reference to sample treatment and analytical techniques.

Sample	Species	Treatment	Technique	Reference
Beans (kidney)	As(III), As(V), DMA, MMA	Extraction with 2 mol/L trifluoroacetic acid at 100 °C, 6 h	HPLC-HG-AFS	[93]
Bean (green, mung)	Cr, Mo and W	Liquid–liquid extraction/separation with membrane	ICP-OES	[147]
Bean (white seeds)	Cd, Co, Cu, Fe, Mn, Mo, Ni, P, S, Se, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[168]
Beans	i-As	Acid digestion: solvent extraction with HCl digestion overnight, reduction with hydrazine-HBr, extraction with $CHCl_3$ (three times), back-extraction to HCl phase (twice), followed by dry ashing with ashing aid at 450 °C	FI-HG-AAS	[154]
Pea seeds	Co, Cu, Fe, Mn, Mo, Ni, P, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[169]
Peanut	Cu, Mn, Ni Zn	Extraction with 0.1 mol/L NaOH vortex, 30 min	SEC-UV-ICP-MS	[181]
Soybean flour	Cd, Co, Cu, Fe, Mn, Mo, Ni, P, S, Se, Zn	Extraction with 0.02 mol/L Tris-HCl, 1 h	SEC-ICP-MS	[168]

Chromatograph-based speciation has been mainly employed, followed by selective extraction and other non-chromatographic methods based on the use of proportional equations to differentiate inorganic and organic species and to check different oxidation states for which the capability of hydride generation of the target elements can be controlled. In order to provide a picture of the present state of speciation of minerals in cereals and pulses, the total content of As, Se and other elements are summarized in Figures 22.5, 22.6 and 22.7, together with a relation description of the different species identified and quantified in rice, wheat and other cereals and beans.

Arsenic is by far the most studied element and speciation of As(III), As(V), dimethylarsenic acid (DMA) and monomethylarsenic acid in rice produced in different areas around the world and also in wheat and wheat-based products, like bread, pasta or semolina, has been examined. It should be noted that arsenobetaine has been determined in only two studies on rice [16, 146], As(III) or DMA being the species with the highest concentration in rice, followed by As(V).

Several non-chromatography studies have focused on the determination of total inorganic arsenic and, to a lesser extent, total organic arsenic. With regard to sample origin and its influence on the presence of As species in rice, it seems that rice from the USA is richer in methylated arsenic than rice produced in Europe and Asia [15]. Based on the studies by Zavala *et al.* [95], this may be explained by the fact that US rice has a higher total As content than that found in other countries. Additional research has identified that there are two different types of rice, inorganic arsenic type and DMA type, with grains of the former able to transport inorganic As present in water and soil and grains of the latter able to transform these species into DMA [17]. However, Zhao *et al.* [15] discussed the ability of the plants to transform As, showing that methylated As species in rice are derived from the soil. In fact, it seems well demonstrated that rice is a significant source of inorganic As for humans and that the speciation of As, Ge and Si in rice grains could be the key to elucidating the mechanism of arsenate transportation, and thus there is special interest in the use of high-sensitivity and high-resolution instruments [176].

Selenium, selenomethionine (SeMet), Se(VI), selenocystine and, to a lesser extent Se(IV), have been demonstrated (Figure 22.6), the differentiation between total inorganic Se and total organic Se also being reported [52]. This shows the predeominance of total organic Se, especially as SeMet, the most bioavailable species in human intake [31]. The concentration of Se in these samples ranged from 9 ng/g [146] to 99,100 ng/g [75], with high values around hundreds of nanograms per gram in many cases.

Total inorganic Hg and total organic Hg have been determined in rice and wheat flour [94], with concentrations being of the same order for both types of compounds. On the other hand, studies with Sb showed that total concentration was between 0.3 and 2.9 ng/g, with concentrations of Sb(III) and Sb(V) of the same order of magnitude. With regard to Te, total concentration was between 0.9 and 20 ng/g, concentrations of Te(VI) being slighly higher than those of Te(IV).

In short, it can be concluded that additionall speciation studies are required in order to clearly identify the mechanism of absorption of minerals from soil and water, and the detoxification pathways. In addition, it would be useful to extend these studies to other elements like Cr and Fe and chemical species such as arsenosugars to complete our knowledge on the presence of essential and toxic elements in important foods.

22.5 Challenges in the mineral composition of cereals and pulses

In-depth study of the mineral profile of cereals and pulses is mandatory based on both their importance in the human diet and the continuous increase in production of rice and wheat worldwide, related to the constant growth of the population. Thus in the near future there is likely to be an increase in studies related to the mechanisms of absorption of minerals from soil and water by the plants and the bioavailability of species for humans, especially with regard to correct evaluation of the appropriate areas for cultivation and to clearly identify the risks of using previously unemployed soils.

Another important issue related to cereals concerns the possibility of the enrichment of these basic foods by minerals traditionally deficient in the diets of some areas, in order to prevent illness and to improve the health of the population, especially those where diets are poor in fish, meat and fresh vegetables. However, in order to achieve this, multi-element and speciation studies are required to provide a complete picture of the benefits of

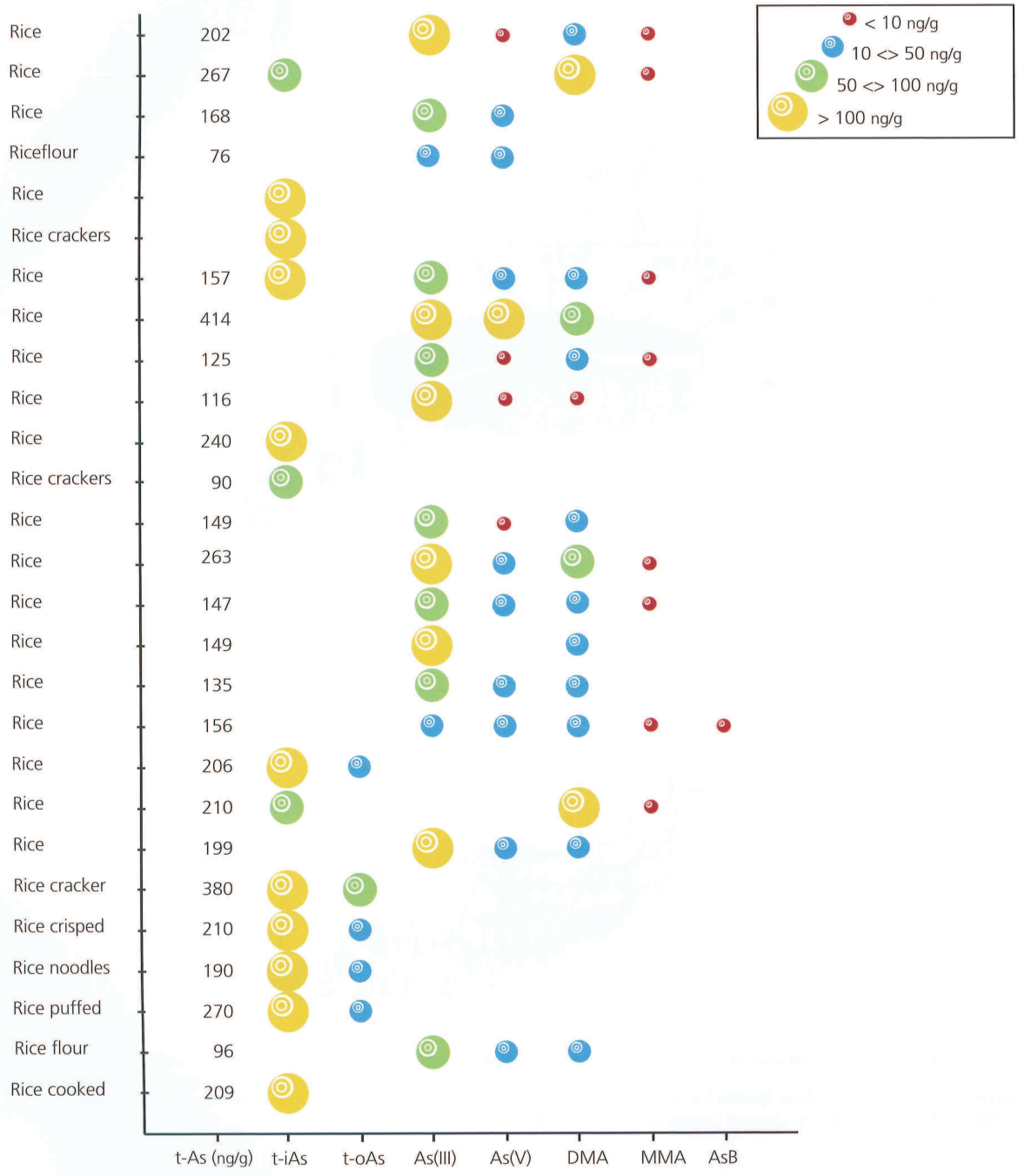
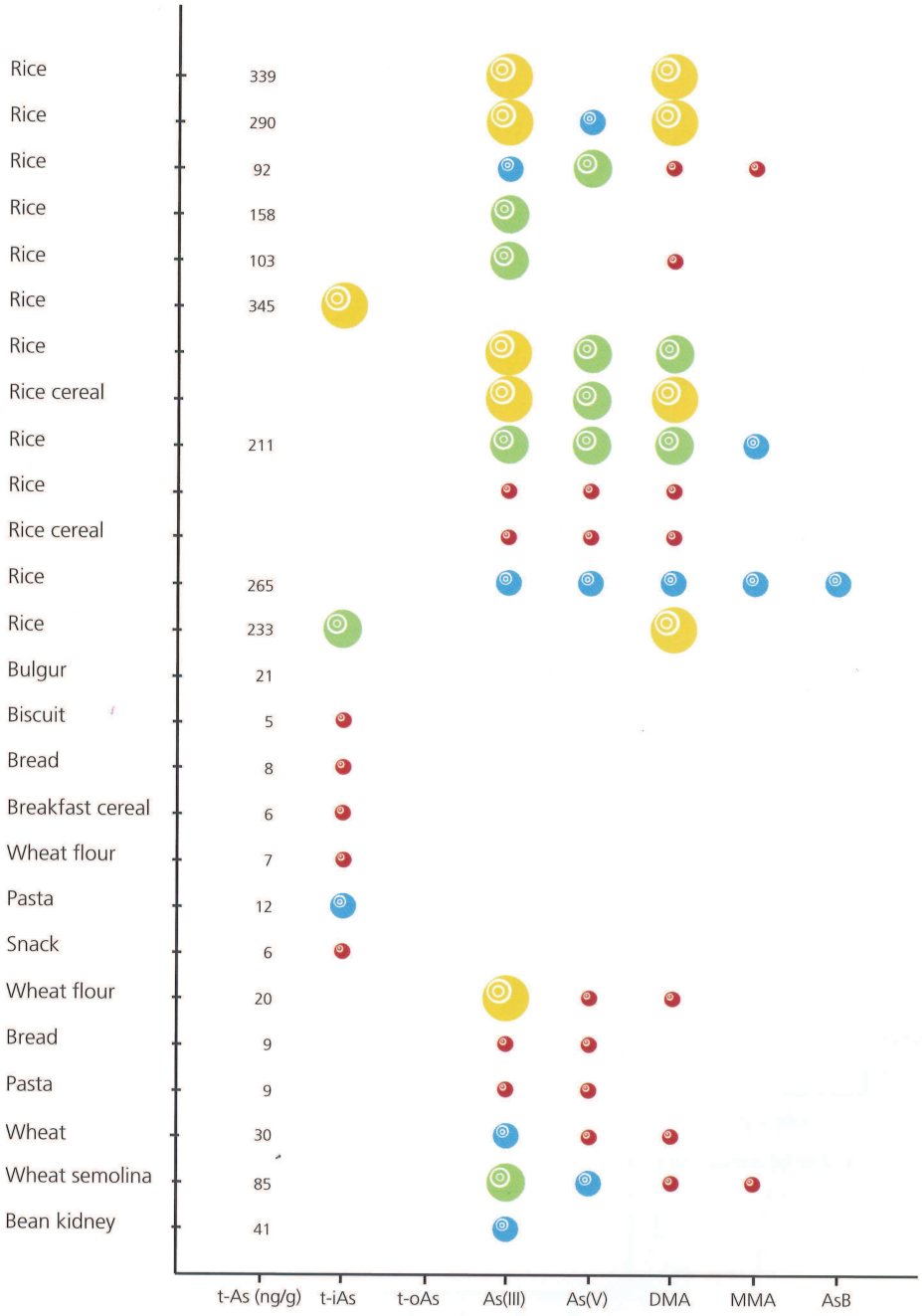


Figure 22.5 Speciation of As in cereals and pulses.



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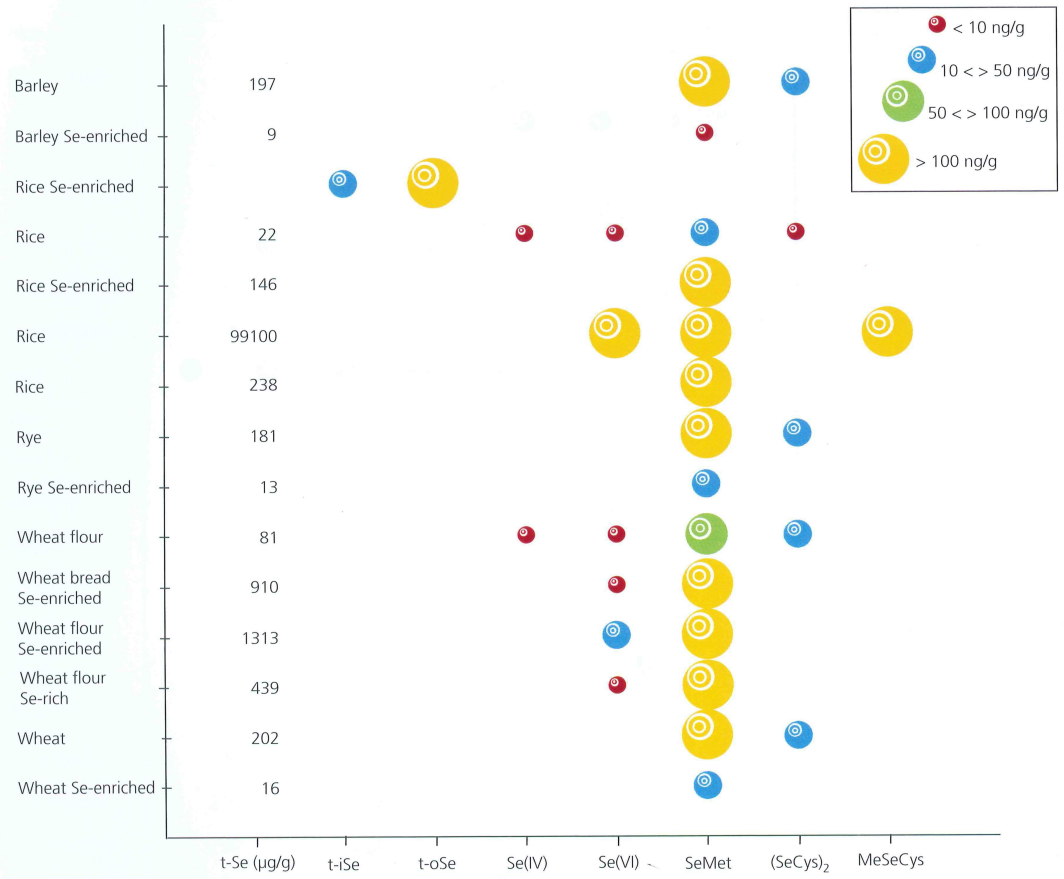

Figure 22.6 Speciation of Se in cereals and pulses.

adding essential elements to wheat and rice, in particular an evaluation of their practical absorption in the human body because metabolic pathways depend on their chemical forms and the presence of other elements.

To guarantee the safety of cereals and pulses and to correctly adjust the addition of minerals to these basic foods, it is essential that there are new developments in methodologies that increase their sensitivity and selectivity, a serious improvement in demonstrating speciation at low concentrations, and an increase in application studies providing data on cereals and pulses produced from areas with different soils and water.

Abbreviations

AAS: Atomic absorption spectrometry
AHG-ICP-MS: Automated hydride generation inductively coupled plasma mass spectrometry
AsB: Arsenobetaine
ASV: Anodic stripping voltammetry
CFD-ICP-OES: Continuous-flow dialysis inductively coupled plasma optical emission spectrometric
CPE-ETAAS: Cloud point extraction electrothermal atomic absorption spectrometry
CPE-FAAS: Cloud point extraction flame atomic absorption spectrometry
CPE-GFAAS: Cloud point extraction graphite furnace atomic absorption spectrometry
CV-AAS: Cold vapour atomic absorption spectrometry
DASCP: Derivative anodic stripping chronopotentiometry
DMA: Dimethylarsenic acid
DPASV: Differential pulse anodic stripping voltammetry
DRC-ICP-MS: Dynamic reaction cell inductively coupled plasma mass spectrometry
DSPV: Differential surface photovoltage
ET-AAS: Electrothermal atomic absorption spectrometry

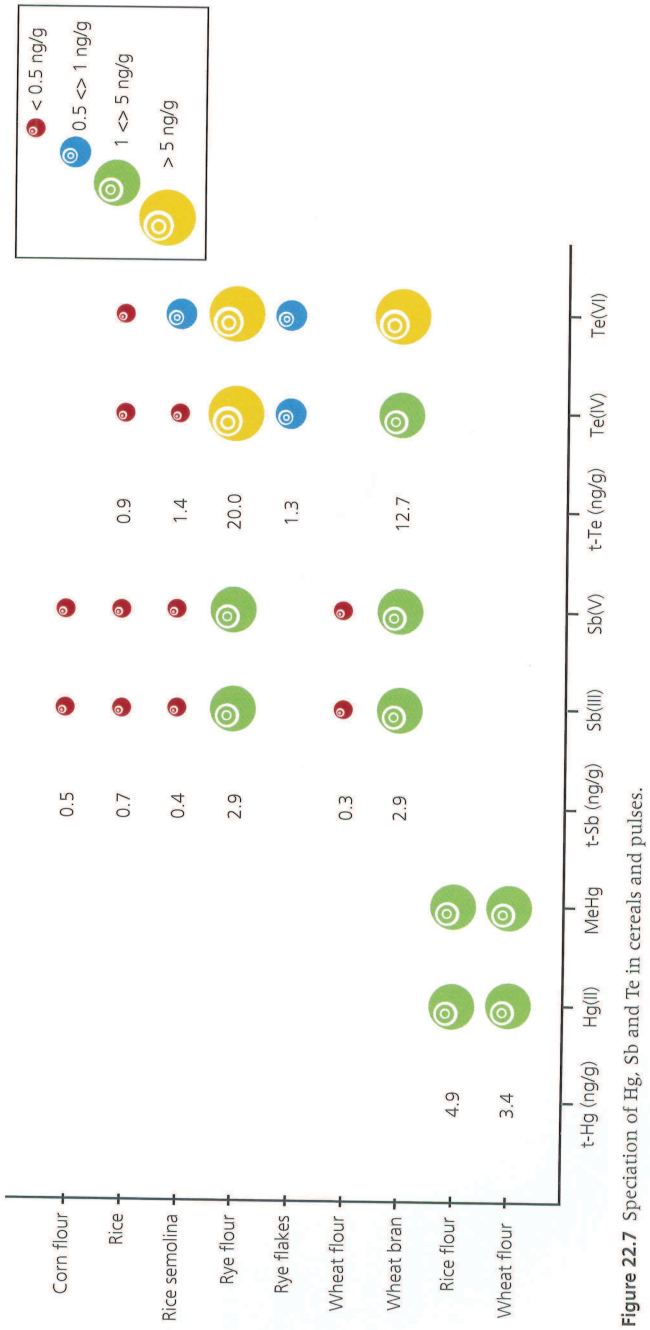

Figure 22.7 Speciation of Hg, Sb and Te in cereals and pulses.

ETV-ICP-OES: Electrothermal vaporization inductively coupled plasma optical emission spectrometry
FAAS: Flame atomic absorption spectrometry
Fe-CAS-ODTA TX100: Complexes of Fe(III) with Chromazurol S (CAS) in the presence of octadecyltrimethylammonium chloride (ODTA) and Triton X-100 (TX100)
FI-CPE-FAAS: Flow injection cloud point extraction flame atomic absorption spectrometry
FI-HG-AAS: Flow injection hydride generation atomic absorption spectrometry
FI-HG-AFS: Flow injection hydride generation atomic fluorescence spectrometry
FI-UV-HG-ICP-MS: Flow injection ultraviolet visible photolysis hydride generation inductively coupled mass spectrometry
FS-FAAS: Fast sequential flame atomic absorption spectrometry
GF-AAS: Graphite furnace atomic absorption spectrometry
HFS-AAS: Hydride generation atomic absorption spectrometry
HG-AAS: Hydride generation atomic absorption spectrometry
HG-AFS: Hydride generation atomic fluorescence spectrometry
HG-ICP-MS: Hydride generation inductively coupled plasma mass spectrometry
HR-CS-GFAAS: High-resolution continuum source graphite furnace atomic absorption spectrometry
HR-ICP-MS: High-resolution inductively coupled plasma mass spectrometry
IC-DRC-ICP-MS: Ion chromatography dynamic reaction cell inductively coupled plasma mass spectrometry.
ICP-MS: Inductively coupled plasma mass spectrometry
ICP-oa-TOF-MS: Inductively coupled plasma orthogonal acceleration time-of-flight mass spectrometry
ICP-OES: Inductively coupled plasma optical emission spectrometry
ICP-ORS-MS: Inductively coupled plasma octopole reaction systems mass spectrometry
INAA: Instrumental neutron activation analysis
IR-MS: Isotope-ratio mass spectrometry
MAD: Microwave-assisted digestion
MCPE: Modified carbon paste electrode
MeHg: Methylmercury
MeSeCys: Se-methylselenocysteine
MMA: Monomethylarsenic acid
MSPE-FAAS: Magnetic solid-phase extraction flame atomic absorption spectrometry
NAA: Neutron activation analysis
PCFFNNs: Principal component-feed forward neural networks
PCINAA: Pseudo-cyclic instrumentation neutron activation analysis
PCRBFNs: Principal component-radial basis function networks
PIXE: Particle-induced X-ray emission
Q-ICP-MS: Inductively coupled plasma quadrupole mass spectrometry
RNAA: Radiochemical neutron activation analysis
RP-HPLC-UV-vis: Reversed-phase high performance liquid chromatography ultraviolet visible spectrophotometry
$(SeCys)_2$: Selenocystine
SeMet: Selenomethionine
SPE-FAAS: Solid-phase extraction flame atomic absorption spectrometry
SPE-GFAAS: Solid-phase extraction graphite furnace atomic absorption spectrometry
SPE-HG-AAS: Solid-phase extraction hydride generation atomic absorption spectrometry
SR-TXRF: Synchrotron radiation total reflection X-ray fluorescence analysis
SS-ETAAS: Solid sampling electrothermal atomic absorption spectrometry
SS-ETV-AFS: Solid sampling electrothermal vaporization atomic fluorescence spectrometry
SS-HR-CS-ETAAS: Solid sampling high-resolution continuum source electrothermal atomic absorption spectrometry
SWASV: Square-wave anodic stripping voltammetry
SWC-SV: Square-wave cathodic stripping voltammetry
SWV: Square-wave voltammetry
t-As: Total arsenic
TDA-AAS: Thermal decomposition, amalgamation and atomic absorption spectrometry
t-Hg: Total mercury
t-iAs: Total inorganic arsenic
t-iSe: Total inorganic selenium
t-oAs: Total organic arsenic
t-oSe: Total organic selenium
t-Sb: Total antimony
t-Se: Total selenium
TS-FF-AAS: Thermospray flame furnace atomic absorption spectrometry

t-Te: Total tellurium
USS-ETV-DRC-ICP-MS: Ultrasonic slurry sampling electrothermal vaporization dynamic reaction cell inductively coupled plasma mass spectrometry
UV/Vis: Ultraviolet/visible spectrophotometry

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1.2. Determinación del perfil mineral de menús infantiles

Mineral profile of fast food children´s menu samples

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Mineral Profile of Children's Fast Food Menu Samples

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Children's fast food menus, including hamburgers served with french fries, dessert, and a soft drink, were analyzed to obtain the mineral profile of trace elements. The developed analytical methodology involved sample digestion under pressure inside a microwave oven with a mixture of HNO_3 and H_2O_2 and inductively coupled plasma-optical emission spectrometry. The method was validated by carrying out the analysis of certified reference materials (NIST 1570a spinach leaves, NCS ZC73016 chicken, and NIST 1568a rice flour) and using recovery experiments. Repeatability was verified by analyzing replicate samples. Twenty-six elements were studied, 12 of which—aluminum, barium, calcium, copper, iron, potassium, lithium, magnesium, manganese, sodium, strontium, and zinc—were quantitatively determined. Results were compared with other studies of fast food and children's menus published in the literature, and the nutritional value of samples was assessed with dietary intake guidelines.

Fast food restaurant chains are ever-more common around the world. They are conveniently located and offer tasty foods at low prices available at any time of day, all characteristics that make these restaurants suitable for modern life styles in developed countries (1). In Spain, governmental data from 2014 indicates that fast food restaurants represent 10.1% of feed expenditures and 31.5% of expenditures on feed outside the home (2), and, because of this, some authors believe the Mediterranean diet is under threat (1). Fast food is commonly associated with a poor diet with high intakes of energy, sodium, and saturated fat (3). Children are high-potential consumers of fast food because they are strongly influenced by marketing and branding (4). Consumption of fast food in children is especially concerning due to the specific nutritional needs for proper development and growth during childhood and because childhood obesity in the 21st century is at pandemic levels (5). In the latest Spanish National Health Survey, 25.1% of 5495 children declared daily or frequent consumption of fast food (6). Several studies indicate that 20–26% of children in Spain are overweight and 7–18% are obese (7, 8). In the scientific literature, many studies are concerned with the nutritional properties in fast food. Most focused on total calorie, saturated fat, protein, carbohydrate, and fiber content, and the major elements found in menus (9–13). However, to our knowledge, there is too little data on the direct evaluation of the mineral profile of complete fast food menus; thus, the goal of this study was to develop a method for the qualitative and quantitative determination of as many mineral elements as possible and evaluate fast food's contribution to the recommended daily intake of minerals in children. In the present study, both toxic and nutrient elements were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) after sample microwave-assisted digestion.

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Material and Methods

Instruments and Apparatus

Determinations were carried out using a dual-viewing Perkin Elmer spectrometer (Model Optima 5300 DV; Norwalk, CT), equipped with a cross-flow nebulizer and Scott spray chamber. A Microwave Labstation Ethos SEL (Milestone, Sorisole, Italy) was used to digest samples. Other equipment included a Trace-Clean automatic cleaning device (Milestone), a Selecta ultrasound water bath (Barcelona, Spain), and a Cryodos 50 lyophilizer (Telstar, Barcelona, Spain). Ultrapure water, with a resistivity of 18.2 MΩ cm, was obtained with a Milli-Q system (Millipore, Bedford, MA).

Reagents and Standards

All chemicals were of the highest purity available and all solutions prepared in ultrapure water. Scharlau 69% HNO_3 and 35% H_2O_2 (Barcelona, Spain) were used for sample digestion and dilution and to prepare standard solutions. Argon C-45 (purity >99.995%) was supplied by Carburos Metálicos (Barcelona, Spain).

For calibration of minor and trace elements, a multielement standard solution of 100 mg/L, containing 26 elements [aluminum (Al), arsenic (As), boron (B), barium (Ba), beryllium (Be), bismuth (Bi), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), Molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr), titanium (Ti), thallium (Tl), vanadium (V), and zinc (Zn)] diluted in 5% HNO_3 (v/v) were used. Additionally, a solution of the major elements Ca, K, Mg, and Na was prepared from corresponding monoelemental standards of 1000 mg/L in 0.5 M HNO_3. Ruthenium (Ru; 1.0 g/L) was supplied by Fluka (Buchs, Switzerland) and used as the internal standard.

Certified reference materials (CRMs) were supplied by the National Institute of Standards and Technology (Gaithersburg, MD) for spinach leaves (NIST 1570a) and rice flour (NIST

1568a) and by the China National Analysis Centre for Iron and Steel (Beijing, China) for chicken (NCS ZC73016).

Sampling, Storage, and Handling

Menu samples used throughout this study corresponded to different restaurants and were obtained on different days from two fast food chains with an extensive presence in Spain. The fast food menus were composed of a hamburger bun, a beef hamburger patty, cheddar cheese, a pickle, ketchup (extra amounts included in menus I–IV and VI), mustard (extra amounts included in menus I and II), regular-size french fries, yogurt (menus I–V) or milk shake (menu VI), and a soft drink [cola (menus I, IV, and VI), orangeade (menu II), lemonade (menu III), or lemon tea (menu V)]. Menus were mixed and homogenized with a conventional mill. For preservation, each menu was freeze-dried for approximately 48 h at a chamber pressure of 0.05 mbar and powdered dry samples stored in plastic flasks until analysis.

Sample Preparation Procedures

For digestion, three 0.5 g subsamples were accurately weighed. A predigestion step was carried out to prevent loss by overpressure during the microwave process: 6.5 mL HNO_3 and 3.5 mL H_2O_2 were carefully added to the samples and the mixture sonicated in an ultrasound water bath for 90 min. Samples were digested in a microwave oven according to the following program: 500 W exit power, 3 min to reach 85°C, 9 min to reach 145°C, 4 min to reach 180°C, and then held for 15 min at 180°C and 25 min at 30°C (14). Digested samples were diluted to a volume of 20 mL with ultrapure water. Before ICP-OES measurement, 0.1 mL of the 10 mg/L Ru solution was added to 9.9 mL digested samples.

ICP-OES Determination

The operating conditions used for ICP-OES determination were as follows: RF power of 1300 W, plasma flow of 15 L/min Ar, auxiliary flow of 0.2 L/min Ar, nebulizer flow of 0.8 L/min

Table 1. Wavelength used in and analytical figures of merit for the developed procedure for the determination of mineral elements in children's fast food menus

Mineral	Wavelength, nm	LOD, μg/g[a]	LOQ, μg/g[b]	Recovery, %[c]	RSD, % (concn, μg/g)[d]
Al	396.153	6.3	21.0	101 ± 13	—
As	188.979	1.9	6.3	96 ± 8	—
B	249.677	2.2	7.0	53 ± 2	—
Ba	233.527	0.13	0.43	106 ± 2	2 (0.745)
Be	313.107	0.20	0.67	99 ± 1	—
Bi	223.061	0.48	1.60	92 ± 1	—
Ca	317.933	26	86	—	5 (2200)
Cd	228.802	0.14	0.47	100 ± 1	—
Co	228.616	0.10	0.33	93 ± 2	—
Cr	267.716	0.27	0.90	112 ± 4	—
Cu	327.393	0.18	0.60	119 ± 7	3 (1.14)
Fe	238.204	1.4	4.7	93 ± 1	2 (13.3)
K	766.49	20	67	—	4 (3860)
Li	670.784	0.03	0.10	143 ± 1	—
Mg	285.213	1.5	5	—	5 (590)
Mn	257.61	0.04	0.13	101 ± 3	2 (2.34)
Mo	202.031	0.19	0.63	118 ± 1	—
Na	589.592	7	23	—	3 (5900)
Ni	231.604	0.19	0.63	96 ± 2	—
Pb	220.353	0.49	1.63	94 ± 2	—
Se	196.026	1.06	3.53	100 ± 4	—
Sr	407.771	0.07	0.23	100 ± 2	2 (5.11)
Ti	334.94	0.08	0.27	119 ± 1	—
Tl	190.801	0.85	2.83	96 ± 5	—
V	290.88	0.93	3.10	107 ± 2	—
Zn	206.200	0.7	2.3	94 ± 5	3 (22.5)

[a] LODs were calculated as concentrations corresponding to signals equal to 3 times the SD of 10 blank solutions, taking into consideration the taking into consideration the mass of digested sample and the volume of diluted sample after digestion (μg/g dry weight).

[b] LOQs were calculated as concentrations corresponding to 10 times the SD of 10 blank solutions, taking into consideration the number of taking into consideration the mass of digested sample and the volume of diluted sample after digestion (μg/g dry weight).

[c] Recovery studies were carried out for actual children's menu samples spiked with a known concentration (5 μg/g). Recovery studies were not carried out for Ca, K, Mg, and Na. Results are the average ± SD of three independent analyses.

[d] Percent RSDs were calculated from the average of the repeatability of three independent analyses carried out on actual samples. Concentration ranges are presented in parentheses (μg/g dry weight).

Ar, sample uptake rate of 1.1 mL min, axial or radial view (the latter only for K, Na, Ca, and Mg), two-point background correction, and a total of three replicates for each analytical measurement. The most sensitive emission line, free of spectral interferences, was selected for each element (*see* Table 1).

Calibration standards were prepared daily by diluting stock standards and adding internal standard. Calibrations for trace and major elements were set at ranges of 0.05–2 and 1–60 mg/L, respectively. Two reagent blanks were subjected to the same treatment than samples for every set of samples and measured together in the same run, to correct analytical signals, if necessary, and control cross-contamination. Additionally, control standards were measured every 10 independent sample measurements.

Results and Discussion

Analytical Features of the Method

Analytical figures of merit, sensitivity, LOD, LOQ, and precision of the method were obtained for the method validation. As Table 1 shows, the LOD values were adequate for the determination of trace elements in the children's menu samples. The RSD obtained for each element in the sample under study was less than 5% for all samples, indicating suitable precision of the method. Analysis of 5 µg/g spiked samples provided acceptable recoveries, varying between 92 and 119%, with the exception of B and Li, which were likely biased by cross-contamination and saturated signals, respectively.

Accuracy was tested by CRM analysis. Comparison between results found and expected was carried out with ratio concentrations; a statistical test was also applied that followed Joint Research Center guidelines (15). Table 2 presents the results of the CRM analysis in which different kinds of foods involved in the determination of each analyte at different concentration levels. According to the statistical test, good recoveries were obtained, with the exception of Al, which was likely biased by the subtraction of the blank that was affected by Al contamination from the alumina injector.

Trace Element Content in the Fast Food Children's Menu Samples

Table 3 reports data that was obtained for the determination of the mineral profile of six menu samples. From the initial 26 elements studied, 12 (Al, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Sr, and Zn) were detected in at least 1 of the analyzed samples. The major elements found were K and Na, with average values of 1580 and 1980 µg/g, respectively; whereas, Ca and Mg had average values of 420 and 80 µg/g. Fe, Zn, and Sr, followed by Cu, Mn, and Ba, were found at as low as the µg/g

Table 2. Analysis of CRMs by using the developed procedure for the determination of the mineral profile in children's fast food menus

	NCS ZC73016 chicken			NIST 1570a spinach leaves			NIST 1568a rice flour		
Mineral	Concn found, µg/g[a]	Certified value, µg/g	Recovery, %[b]	Concn found, µg/g[a]	Certified value, µg/g	Recovery, %[b]	Concn found, µg/g[a]	Certified value, µg/g	Recovery, %[b]
Al	53 ± 13	NR[c]	—	200 ± 50	310 ± 11	65[d]	NQ[e]	4.4 ± 1.0	—
B	NQ	0.76 ± 0.13	—	46 ± 10	37.6 ± 1.0	122[f]	NQ	NR	—
Ba	1.4 ± 0.5	1.5 ± 0.4	93[f]	6.5 ± 1.2	NR	—	NQ	NR	—
Ca	NQ	220 ± 20	—	14000 ± 1800	15270 ± 410	92[f]	126 ± 9	118 ± 6	107[f]
Cd	NQ	0.005	—	2.7 ± 0.6	2.89 ± 0.07	93[f]	NQ	0.022 ± 0.002	—
Cu	1.5 ± 1.5	1.46 ± 0.12	103[f]	15 ± 3	12.2 ± 0.6	123[f]	2.0 ± 0.3	2.4 ± 0.3	83[f]
Fe	32 ± 9	31 ± 3	103[f]	220 ± 40	NR	—	8.14 ± 1.08	7.4 ± 0.9	110[f]
K	14800 ± 160	14600 ± 700	101[f]	27000 ± 3000	29030 ± 520	93[f]	1220 ± 80	1280 ± 8	95[f]
Li	0.041 ± 0.019	0.034 ± 0.007	121[f]	3.6 ± 0.7	NR	—	NQ	NR	—
Mg	1230 ± 16	1300 ± 100	95[f]	8400 ± 1000	8900	94[f]	530 ± 40	560 ± 20	95[f]
Mn	1.3 ± 0.4	1.65 ± 0.07	79[f]	75 ± 15	75.9 ± 1.9	—	19 ± 2	20.0 ± 1.6	95[f]
Mo	NQ	0.11 ± 0.01	—	0.4 ± 0.2	NR	—	1.1 ± 0.4	1.46 ± 0.08	75[f]
Na	1430 ± 20	1440 ± 90	99[f]	17000 ± 2000	18180 ± 430	94[f]	NQ	6.6 ± 0.8	—
Ni	NQ	0.15 ± 0.03	—	1.1 ± 0.2	2.14 ± 0.10	51[f]	NQ	NR	—
Sr	0.56 ± 0.10	0.64 ± 0.08	88[f]	50 ± 7	55.6 ± 0.8	90[f]	NQ	0.38 ± 0.04	—
Zn	32 ± 6	26 ± 1	123[f]	83 ± 16	82 ± 3	101[f]	19 ± 4	19.4 ± 0.5	98[f]

[a] Results are the average ± SD of three independent analyses.

[b] Using $\Delta m \leq U\Delta$, the found concentration and certified values were comparable, where Δm = absolute difference between mean the measured value and the certified value; and $U\Delta$ = expanded uncertainty of the difference between the result and the certified value, calculated as $U\Delta = 2 \times [(SD/n^{1/2})^2 + (U_{CRM}/2)^2]^{1/2}$, where n = number of replicates; and U_{CRM} = expanded uncertainty of each certified value given on the certificate. A structured and quantitative approach was performed, taking into account the certified reference value ($C_{CRM} \pm U_{CRM}$), the mean measured result corresponding to three independent analyses ($C_m \pm SD$), and their respective uncertainties. These uncertainties were subsequently combined, and the expanded uncertainty $\{U\Delta = 2 \times [(SD/n^{1/2})^2 + (U_{CRM}/2)^2]^{1/2}\}$ was compared with Δm values. If the values of Δ_m were lower than $U\Delta$, it is likely that there was no significant difference between obtained values for CRMs and the certified values.

[c] NR = not referenced.

[d] Not acceptable, according to statistical analysis criteria.

[e] NQ = Found values were below the LOQ of the method (*see* LOQ in Table 1).

[f] Acceptable according to statistical criteria.

Table 3. Mineral profile (μg/g fresh weight) of children's fast food menus[a]

Mineral	Menu I (497 g)	Menu II (579 g)	Menu III (565 g)	Menu IV (520 g)	Menu V (529 g)	Menu VI (695 g)
Al	1.81–6.03	1.81–6.03	ND[b]	ND	1.81–6.03	ND
Ba	0.14 ± 0.03	0.16 ± 0.04	0.140 ± 0.005	0.16 ± 0.02	0.16 ± 0.04	0.190 ± 0.004
Ca	382 ± 13	309 ± 9	443 ± 19	455 ± 28	398 ± 4	548 ± 25
Cu	0.75 ± 0.13	0.69 ± 0.09	0.56 ± 0.04	0.69 ± 0.13	0.54 ± 0.02	0.284 ± 0.007
Fe	4.6 ± 0.6	4.2 ± 0.3	5.4 ± 0.5	6.0 ± 1.3	4.2 ± 0.5	3.31 ± 0.07
K	2040 ± 40	1740 ± 40	1550 ± 50	1440 ± 60	1750 ± 20	960 ± 40
Li	0.008–0.029	0.008–0.029	0.008–0.029	0.008–0.029	0.008–0.029	0.008–0.029
Mg	149 ± 3	116 ± 2	136 ± 6	144 ± 6	132.9 ± 1.3	147 ± 7
Mn	0.84 ± 0.13	0.78 ± 0.12	0.72 ± 0.02	0.88 ± 0.13	1.44 ± 0.03	0.583 ± 0.010
Na	1850 ± 70	2260 ± 40	2110 ± 60	2400 ± 100	1800 ± 100	1470 ± 50
Sr	1.39 ± 0.19	0.52 ± 0.06	1.36 ± 0.04	1.5 ± 0.2	1.23 ± 0.02	1.27 ± 0.03
Zn	7.5 ± 0.6	6.9 ± 0.9	7.9 ± 0.2	8.0 ± 0.2	6.11 ± 0.10	5.6 ± 0.2

[a] Data are in fresh weight, taking into account average moistures of 68, 71, 69, 69, 74, and 75% in menus I–VI, respectively. Results of the quantifiable elements are expressed as the average ± SD of three independent analyses. Results of nonquantifiable elements are expressed as detectable ranges. Elements below the LOD in all menus (μg/g fresh weight) were as follows: As < 0.54, B < 0.60, Be < 0.06, Bi < 0.14, Cd < 0.04, Co < 0.03, Cr < 0.08, Mo < 0.05, Ni < 0.05, Pb < 0.14, Se < 0.3, Ti < 0.02, Tl < 0.24, and V < 0.27.

[b] ND = Not detected.

Table 4. Data reported in the literature concerning the mineral composition of fast foods items and fast food

Fast food	Country	Data source or method	Mineral element content[a]	Ref.
		Items		
Kids menu food items, including entrees and side dishes	Australia, Canada, New Zealand, United Kingdom, United States	Food company	Na (0–1010 mg per item)	(3)
Kimbap, tteokbokki, sandwiches, and hamburgers	Korea	ICP-OES	Na (23–1547 mg per serving)	(23)
Meat pies, doughnuts, moin moin (steamed bean pudding), and cake	Nigeria	AAS[b]	Co (ND), Cu (ND), Fe (119–327 μg/g dry weight), and Zn (323–1071 μg/g dry weight)[c]	(22)
Beef-, chicken-, fish-, and pork-based products; as well as samples containing egg as a major constituent and several types of sauces	Spain	ET-AAS[d]	Ni (18.5–95.0 ng/g), Cr (0.01–1.10 μg/g), Mn (0.15–2.90 μg/g), and Al (0.85–38.10 μg/g)	(16, 20)
Burgers, nuggets, french fries, and sandwiches	Bahrain	F-AAS[e]	Ca (337–1893 μg/g), Cu (5.4–11 μg/g), Fe (3.3–82.9 μg/g), K (1491–2559 μg/g), Mg (218–487 μg/g), Mn (0.27–3.75 μg/g), Na (4315–7890 μg/g), and Zn (4.9–22.7 μg/g)	(19)
Food and food products that contain Al as a food additive	United States	ET-AAS	Al (1–2.7%)	(17)
Hamburgers, cheeseburgers, fish burgers, chicken sandwiches, fried chicken strips, pizza, french fries, and apple pie	Poland	ET-AAS	Cr (3.76–28.6 μg 100 g) Ca (10–192.2 mg per serving), Fe (0.6–2.3 mg per serving), and Mg (5.9–37.3 mg per serving)	(18)
		Menus		
Kids menus (McDonald's, KFC, Hungry Jack's, Chicken Treat, and Red Rooster, Oporto)	Australia	Food companies	Na (667–737 mg per menu)	(12)
Fast food menus (excluding drinks)	United Kingdom	Nutrient databases	Ca, Na, Fe, Zn, (168.8, 510, 1.2, 1.1 mg per menu)	(25)
Kids menus, including burgers, chicken, deli, tacos, hot dogs, and sandwiches (excluding drinks)	United States	Food companies and nutrient databases	Ca (197.1 ± 167.5 mg per menu), Fe (2.2 ± 1.4 mg per menu), and Na (862.9 ± 288.4 mg per menu)	(24)

[a] Data in parentheses are the determined mineral element concentrations.

[b] AAS = atomic absorption spectroscopy.

[c] ND = not detected.

[d] ET = electrothermal.

[e] F = flame.

Table 5. Comparison between the mineral composition (mg/menu) of children's fast food menus and other menus

Menu	Ca	Cu	Fe	K	Mg	Mn	Na	Zn	Ref.
Children's fast food[a]	179–382	0.20–0.40	2.2–3.1	670–1010	67–102	0.41–0.76	920–1310	3.2–4.4	This study
Children's fast food[b]	197.1 ± 167.5	—	2.2 ± 1.4	—	—	—	862.9 ± 288.4	—	(24)
Children's fast food[b]		—	—	—	—	—	400–1454	—	(12)
Fast food[b]	168.8 ± 143.1	—	1.2 ± 0.4	—	—	—	510.8 ± 257.6	1.1 ± 0.7	(25)
Preferred by children[b]	113 ± 101	0.29 ± 0.09	2.4 ± 1.2	719 ± 254	56 ± 19	0.48 ± 0.24	888 ± 619	3.4 ± 2.3	(28)
Scholar[b]	199.3 ± 106.2	—	5.72 ± 3.13	1065.7 ± 415.9	—	—	461.6 ± 434.7	3.61 ± 2.60	(26)
Scholar[b]	477 ± 6	—	4.40 ± 0.05	—	—	—	1348 ± 21	—	(27)
Scholar[b]	—	—	—	—	—	—	935–1327	—	(29)
Canteen[a]	264–528	0.024–0.106	1.48–7.98	940–3807	98–382	0.640–1.920	1464–4848	3.1–6.8	(14)

[a] Data obtained by direct analysis of menu samples using chemical analytical techniques.

[b] Data obtained from fictitious menus and from nutrient databases and company information.

and ng/g level, with a maximum average of 3 μg/g for Fe and a minimum average of 0.1 μg/g for Ba. Finally, Al and Li could only be approximately estimated because, even though they were detected, they were under the LOQ of the method.

The aforementioned results for the complete menus could not be directly compared with the mineral composition of the fast food items presented in Table 4 that provide data from the analysis of fast food items (16–23) and fast food menus from nutrient databases and food company Web sites (3, 12, 24, 25). However, it is likely that nondiscordant results were obtained as a result of experimental and reported data being in the same order of magnitude for each considered element, the only exceptions being data from Nigerian fast food products, which were significantly high (22), and data provided for food products containing Al as a food additive (17).

In Table 5, the results in the present study were compared with those obtained from the analysis of Valencian canteen menus (14), fictitious fast food menus, and fictitious scholar menus obtained from nutrient databases and from company-provided information (26–29). As can be seen in Table 5, in general, the values we obtained were very close to those found in other fast food menus and the menus preferred by children. It should also be noted that Fe and K content in fast food menus was lower than the values found in scholar and canteen menus.

Dietary Intake of Infants Consuming Fast Food

To assess the data obtained in our present study from a nutritional point of view, we calculated dietary intake for each element representing one menu and compared the results with Dietary Reference Intake (DRI) recommendations for children and young adults 5–20 years old, as recommended by different organizations. Figure 1 shows the percent DRI from the analyzed children menus. Adequate values were found, corresponding to about 30–40% for most elements. However, the presence of Fe and K in analyzed samples was around 20% of the recommended daily intake, which is too low. Thus, these dietary requirements should be supplemented through other kind of foods during the day. On the other hand, the Na content, which was as high as 70% of the DRI, was too high and attributed to sodium chloride added during the cooking process.

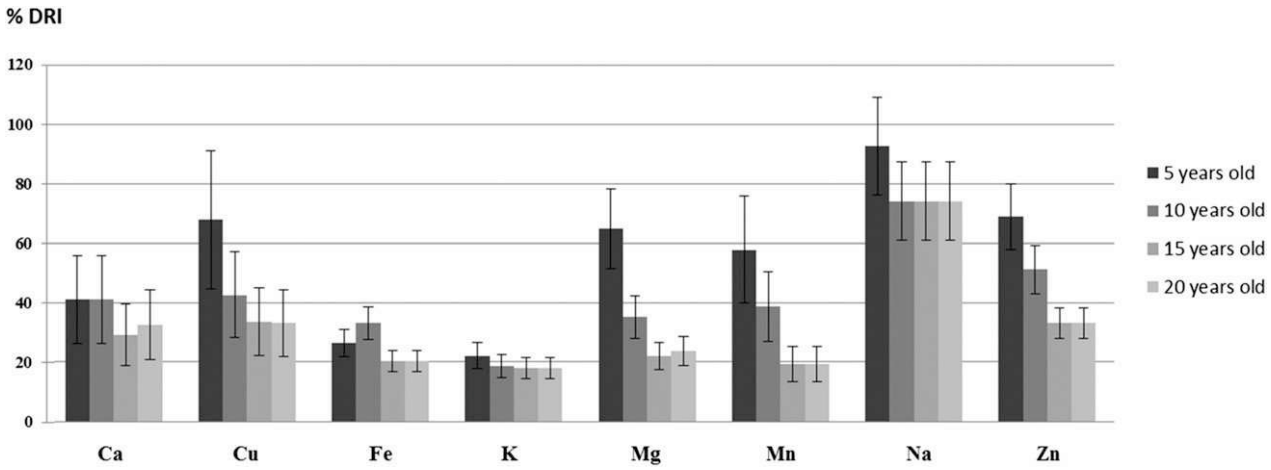

Figure 1. Comparison of mineral concentrations found in children's fast food menu samples per DRI recommendations. *Note*: DRI is the dietary requirement for Ca and adequate intake of Mn and Zn as recommended by the European Food and Safety Authority, whereas for Cu, Fe, and Mg, the recommended dietary allowances are from DRI; for K and Na, the adequate recommended intake is from the Institute of Medicine (The National Academy of Sciences, Engineering, and Medicine). Ranges were as follows: dietary requirement of 680–860 mg/d for Ca (30), adequate intake of Mn and Zn estimated as 1–3 and 5.5–16.3 mg/d, respectively (31, 32); Cu, Fe and Mg recommended dietary intake ranged from 0.4 to 0.9, 10 to 13, and 130 to 355 mg/d, respectively; and K and Na adequate intake ranged from 3800 to 4700 and 1200 to 1500 XXX, respectively (33).

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1.3. Determinación del perfil mineral del panga

Fast determination of fish mineral profile. Application to Vietnamese panga fish

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ABSTRACT

A methodology, based on inductively coupled plasma optical emission spectrometry after microwave-assisted digestion with HNO_3/H_2O_2, was developed to determine the mineral profile of panga fish samples imported from Vietnam. A total of 42 essential and toxic elements were studied in seven samples taken from the local market. Preliminary studies were focused on selecting the best wavelength and the required dilution of samples in order to provide the highest sensitivity to maximize the number of analytes to be determined without spectral or matrix interferences. Adequate accuracy was assured by the analysis of certified reference material TORT-2. Mercury was also determined by a direct method based on atomic absorption spectrometry. Results obtained indicated a low mineral profile, fourteen elements were found at quantitatively levels, Na (6000 $\mu g\,g^{-1}$) K (1800 $\mu g\,g^{-1}$) Mg (173 $\mu g\,g^{-1}$), Ca (80 $\mu g\,g^{-1}$), Zn (2.44 $\mu g\,g^{-1}$), Fe (1.6 $\mu g\,g^{-1}$), Al (1.1 $\mu g\,g^{-1}$), Sr (0.4 $\mu g\,g^{-1}$) and B, Ba, Hg, Mn, V (under 0.1 $\mu g\,g^{-1}$). Additionally data were compared with those previously reported in literature and an estimation of daily intake was calculated and compared with recommended or tolerable guidelines values. Levels of As, Cd, Pb and Hg were far below the established values by the European Community.

1. Introduction

Pangasius hypophthalmus (Sauvage 1878), commonly referred as panga is a freshwater fish native of South East Asia. It is a highly migratory riverine fish species that makes long-distance migrations over several hundred kilometres. Mature fish can reach a maximum standard total length of 130 cm and up to 44 kg in weight. This species is benthopelagic, typically living within the ranges of pH 6.5–7.5 and temperature 22–26 °C. As an omnivorous specie, panga consumes rice bran, soy and fish by-products during rearing (FAO Fisheries and Aquaculture Department).

During the last decades the capture of wild *Pangasius* seed for aquaculture stocking has been entirely replaced by the stocking of hatchery-produced seed, especially in the Mekong Delta River, in Vietnam. In fact, this can be considered as an icon of aquaculture development with a production around 1 million tonnes at year and exports to over 80 countries, mainly to the European Union (158,209 t of *Pangasius* fillet were imported during 2011) (FAO Globefish, 2012).

The panga fish growing popularity among the consumers worldwide is related to its low price, mild taste and texture and pleasing presentation into filets with no skin or spines. panga fish is considered an affordable 'white fish' and it is specially used in schools, cantinas and hospitals, thus substituting traditional species such as cod, sole or hake. However, since the beginning of the sector growth, it has been subjected to a greater degree of public scrutiny and has provoked criticisms in terms of food safety, environmental performance and social equity, with associated negative media coverage, (OCU, 2010; Fish Information & Services, 2010; Greenpeace, 2012; Little et al., 2012). Although most of these publicities have been unfounded thus far (Phan et al., 2009; AESAN, 2011a), negative perceptions are difficult to change once in the public domain and efforts should be done to increase available scientific data.

Studies on panga fish reported in the literature are scarce and frequently concern to farming practices (Da et al., 2013), and environmental water and soil conditions (Keenan et al., 2010; Udomchoke et al., 2010). Regarding nutritional value and food safety some articles deal with fatty-acids, PAHs and PCBs (Orban et al., 2008; Karl et al., 2010; Szlinder-Richert et al., 2011), but only few publications report data of metal content in fish. Table 1 summarizes the published studies for mineral component determination in Asian panga fish, classified as a function of the analytical methods employed to determine the studied elements. As can be seen the scientific literature only provides data about the major alkaline and alkaline earth elements and eight trace elements, probably due to the use of typical mono-elemental techniques like atomic absorption spectroscopy.

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The use of inductively coupled plasma optical emission spectroscopy (ICP-OES) under appropriate conditions offers the possibility of obtaining multielemental information on the samples over wide concentration ranges, including both, harmful and essential elements, with a low consumption of sample, reagents and time. On the other hand, determination of mercury could be particularly relevant due to its marked tendency to be bioaccumulated and biomagnificated through food chain. The use of a direct sample analysis method (DMA) which is based on thermal decomposition of solid samples followed by amalgamation of the vapour with gold and atomic absorption spectrometry detection allows getting accurate data in a very simple way.

The aim of the present study has been to develop and validate a simple, sensitive and fast analytical procedure, based on ICP-OES after microwave assisted digestion of panga fish samples to determine their mineral profile. Additionally DMA was used to determine Hg in samples. The aforementioned methodologies were applied to the analysis of panga fish samples from Vietnam available in the Valencian market and results were compared with those previously reported and were evaluated in terms of food safety.

2. Material and methods

2.1. Apparatus

Lyophilisation of samples was carried out using freeze dried systems Cryodos 50 and LyoAlfa Plus 10–55 both from Telstar (Barcelona, Spain).

Digestions were performed using a microwave labstation Ethos SEL from Milestone (Sorisole, Italy), equipped with a temperature feedback control. The system was operated at a maximum power of 1000 W. High pressure Teflon containers of 100 mL inner volume were cleaned with sub-boiling HNO_3 by using an automatic cleaning device Trace-Clean from Milestone. An ultrasound water bath from Selecta (Barcelona, Spain), of 9 L volume operated at 50 W power and 50 Hz frequency, was employed for sample sonication.

Measurements by ICP-OES were performed using a dual-viewed spectrometer Perkin Elmer model Optima 5300 DV (Norwalk, CT, USA) with an auto sampler A-93-plus. An ultrasonic nebulizer CETAC U6000AT+ (Omaha, NE, USA) and a cross-flow nebulizer were used during this work. Mercury analysis was made using a direct mercury analyser DMA-80 from Milestone.

Ultrapure water, with a resistivity of 18.2 MΩ cm^{-1} was obtained from a Milli-Q system Millipore (Bedford, USA).

2.2. Reagents

All chemicals used were of the highest purity available and all solutions were prepared in ultrapure water. HNO_3 69 percent (w/w) and 35 percent (w/w) H_2O_2, both from Scharlau (Barcelona, Spain) were employed in sample treatment and dilution.

Standard solutions for calibration were prepared from ICP 26-multielemental standard containing Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V, Zn at a concentration level of 100 mg L^{-1} in 5 percent HNO_3, a 16-lanthanide standard mixture containing Ce, La, Nd and Pr 100 mg L^{-1} and Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y at 20 mg L^{-1} concentration in 5 percent HNO_3, and uni-elemental standard solutions of 1000 mg L^{-1} for major elements Ca, K, Mg, Na, all from Scharlau. A ruthenium solution of 1000 mg L^{-1} from Fluka (Buchs, Switzerland) was used to add the internal standard.

Argon C-45 (purity higher than 99.995 percent), employed as plasmogen and carrier gas in the ICP-OES system, and oxygen employed, in the DMA-80 system were supplied by Carburos Metálicos (Barcelona, Spain).

2.3. Sample collection and preparation

Seven panga fish samples, all of them, imported from Vietnam, were acquired as a deep- frozen or rather fresh filet in different Valencian (Spain) supermarkets during winter 2011. Samples were defrozed, washed with milli-Q water and homogenized using a conventional food mill. Then one portion was preserved without additional treatment and stored in the fridge; and other portions were freeze-dried for approximately 48 h at a chamber pressure of 0.05 mbar and preserved into desiccator until their analysis.

2.4. Sample treatment

For panga fish digestion, 1.5 g of fresh sample, or 0.5 g of lyophilized sample were weighted inside a pre-cleaned teflon vessel, 4 mL of concentrated nitric acid were added and the mixture was sonicated in an ultrasound water bath for 30 min; 2 mL of hydrogen peroxide was carefully added and, 10 min later, the mixture was sonicated during 15 min. A final volume of the samples was made to 10 mL using ultrapure water. The vessels were placed inside the microwave oven and according to the manufacturer suggestions (Milestone, 2006) the following program was run: 500 W exit power, 3 min to reach 85 °C, 9 min to reach 145 °C, 4 min to reach 180 °C, 15 min at 180 °C and 25 min at 30 °C. After cooling, the vessels were placed again in the ultrasound water bath in order to remove nitrous vapours and the resultant solutions were transferred to plastic flasks and diluted to 20 mL final volume.

2.5. ICP-OES determination

Samples were analyzed by ICP-OES using the experimental conditions summarized as: 1300 W RF power, 15 L min^{-1} plasma flow, 0.2 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, 1.1 mL min^{-1} sample uptake rate, axial and radial (Ca, K, Mg, Na and Sr) view, two-point background correction, three number of replicates and cross flow nebulizer. Most elements were measured at two analytical emission lines for comparative purposes. The calibration standards were prepared from stock ones in 1 percent (v/v) HNO_3; Ruthenium 1 mg L^{-1} was used as internal standard. The calibration ranges for the evaluated trace level elements were from 0.02 to 0.4 (lanthanides) or 0.05 to 2 mg L^{-1}; and for the highly concentrated elements (Ca, K, Mg, Na) till 80 mg L^{-1}. Analyte concentrations were determined using external calibration.

2.6. Direct mercury analysis

An amount of 0.2 g fresh samples were weighted inside nickel boats and introduced inside the DMA-80 system to be subjected to thermal combustion in a continuous flow of oxygen following the program: drying 60 s at 250 °C, thermal decomposition 180 s at 750 °C. Hg was determined using atomic absorption at 253.7 nm. The instrument was previously calibrated using aqueous Hg standards in the ranges 0–20 ng Hg and 20–1000 ng Hg, and the calibrations were verified using solid standard reference materials.

2.7. Quality assurance

Each sample was prepared and measured in triplicate. Blank digestions were subjected to the same treatment for every set of samples and measured together.

Table 1
Methods proposed in the literature for the determination of mineral element composition of panga fish.

Technique	Sample pre-treatment	Fish type	Fish origin	Elements determined	Reference
AAS	Microwave digestion with HNO_3	Catfishes	Vietnam	Pb, Cu, Cd	Phanwichien et al. (2010)
AAS		*Pangasius pangasius*	Bangladesh	As, Pb, Cd, Mn	Zaman et al. (2010)
AAS	Hot digestion with $HNO_3/HClO_4/H_2SO_4$	*P. Pangasius*	India	Pb, Cd, Cr, Mn, Zn, Cu	Adhikari et al. (2009)
CV-AAS	Microwave digestion with HNO_3	*Pangasius hypophtalmus*	Vietnam	Hg	Ferrantelli et al. (2012)
CV-AAS	Hot digestion with $H_2SO_4/HNO_3/V_2O_5$	*P. Pangasius*	India	Hg	Pal et al. (2011)
ETAAS, CV-AAS	Microwave digestion	*P. hypophtalmus*	Vietnam	Pb, Hg, Cd, As, Cr	Esposito et al. (2010)
ETAAS, AMA	Microwave digestion with HNO_3	*P. hypophtalmus*	Vietnam	Cd, Pb, Hg	Szlinder-Richert et al. (2011)
ICP-OES, AMA	Microwave digestion with HNO3	*P. hypophtalmus*	Malaysia	As, Cd, Cr, Cu, Hg, Pb, Zn	Mok et al. (2012)
IELC, IAA	Microwave digestion with HNO_3/H_2O_2	*P. hypophtalmus*	Vietnam	Na, K, Mg, Ca, Hg	Orban et al. (2008)

AAS: Atomic Absorption Spectrometry; IELC: Ion Exchange Liquid Chromatography; IAA: Instrumental Activation Analysis; ET-AAS: Electrothermal Atomic Absorption Spectrometry; AMA: direct mercury analysis; CV-AAS: Could Vapor Atomic Absorption Spectrometry; ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy.

Additionally, control standards were measured every ten independent sample measurements.

The limit of detection (LOD) was estimated as the analyte concentration that give signals equivalent to three times the standard deviation of ten blank signals and taking into consideration the amount of sample digested, the final dilution employed in the recommended procedure and the mean moisture content. The limit of quantification (LOQ) was determined in the same way for a factor of ten times the deviation of blank measurements. Instrumental repeatability was established from the average relative standard deviation (RSD) of ten analyses made in 100 ng g^{-1} or 10 μg g^{-1} (Ca, K, Mg, Na) standard solution and repeatability of the whole method was calculated for three independent analyses of real samples employed through this study. Possible matrix effect provided by lyophilization of samples was evaluated by comparing the slope of the standard addition method with the slope of external calibration lines.

Reference material TORT-2 (Lobster Hepatopancreas) from the National Research Council (Ottawa, Canada) was employed in order to making a statement on the evidence of any bias. A structured and quantitative approach was used, taking into account the certified value (C_{CRM}), the measurement result (C_m) and their respective uncertainties. These uncertainties were subsequently combined and the expanded uncertainty (U_Δ) was compared to the difference (Δ_m).

3. Results

3.1. Evaluation of the analytical methodology

Cross-flow and ultrasonic nebulizers were used for the determination of the mineral profile of panga fish samples. The slopes of standard addition method calibration lines were compared with the slopes of external calibration ones; Fig. 1 indicates the ratio between these two values for all the elements considered and using ultrasonic and cross flow nebulizers, with and without using Ru as internal standard. A study of different dilution factors: 1/10, 1/5, 1/2 and the direct measurement of undiluted digested sample was applied using both nebulizers, Table 2 (and Supplementary 1) shows the mineral content of the detected elements in one selected panga fish sample. On the other hand, it was tested the possibility of making ICP-OES determination in digested lyophilized samples in order to increase the number of detected elements. Different dilution factors after sample digestion: 1/5, 1/2 and undiluted ones, were investigated again. Results are shown in Table 2 (and Supplementary 2).

Additionally to verify sensitivity, baseline correction and possible inter-element or matrix interference, all studies were carried out at two wavelength lines for the main part of elements.

The main analytical characteristics of the developed ICP-OES procedure are indicated in Table 3. The sensitivity of the proposed method was evaluated in terms of calibration slope. Detection limits from 0.0014 μg g^{-1} to 0.20 μg g^{-1} for lanthanides and B, Be, Cd, Co, Cu, Li, Mn, Mo, Ni, Pb, Sr, Ti and V; from 0.24 μg g^{-1} to 1.0 μg g^{-1} for Al, As, Bi, Mg, Se and Tl; and from 1.3 μg g^{-1} to 59 μg g^{-1} for Ca, Cr, K, Fe and Na; with RSD values in real samples lower than 30 percent for trace elements and 10 percent for major elements.

3.2. Mineral content in panga fish samples

From the forty two studied elements, thirteen elements (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Sr, V, Zn) were quantitatively determined in seven samples acquired at Valencian market and imported from Vietnam; for the rest of elements the contents found were, in all samples, lower than the LOD values. Table 5 shows the medium values obtained for three independent analysis of each one of the lyophilized samples and expressed as micrograms per gram of fresh sample. A mean moisture content of 82 ± 3 percent was used to transfer data express in dry mass to μg g^{-1} of fresh mass. As can be seen Na (at concentration levels from 2600 till 11300 μg g^{-1}) followed by K (from 1010 to 2490 μg g^{-1}), Mg (from 153 till 180 μg g^{-1}), Ca (from 55 till 120 μg g^{-1}), Zn (from 2.27 to 2.61 μg g^{-1}) and Fe (from 0.51 till 3.9 μg g^{-1}) are the main elements present in the analysed panga fish samples, being found Al at concentration level of 1 μg g^{-1} and the rest of elements at concentration levels in the order of 100 ng g^{-1}.

3.3. Mercury concentration in panga fish

Mercury was directly determined in fresh panga samples. Previous studies were focused on the amount of sample range 100 till 500 mg and it was concluded that there is not any dependency between the amount of sample and the obtained concentration of mercury in this range. So 200 mg of sample were selected as an adequate amount in terms of both, reproducibility and representativity, to obtain appropriate analytical signals. Concentrations found for replicate analysis of the seven samples of panga fish are shown in Table 5 and ranging from 1.52 to 3.9 ng g^{-1}. Additionally it must be noticed that values of 0.280 ± 0.057 μg g^{-1} were obtained for a certified material TORT-2 0.270 ± 0.060 μg g^{-1}.

4. Discussion

4.1. Evaluation of the analytical methodology

In ICP-OES emphasis is given to the problems created by the matrix on the emission measurements of sample elements. Matrix composition could strongly affect the analytical signal because it could modify several parameters: the characteristics and transport of the aerosol generated by the nebulizer, the chemical state in which the analyte is

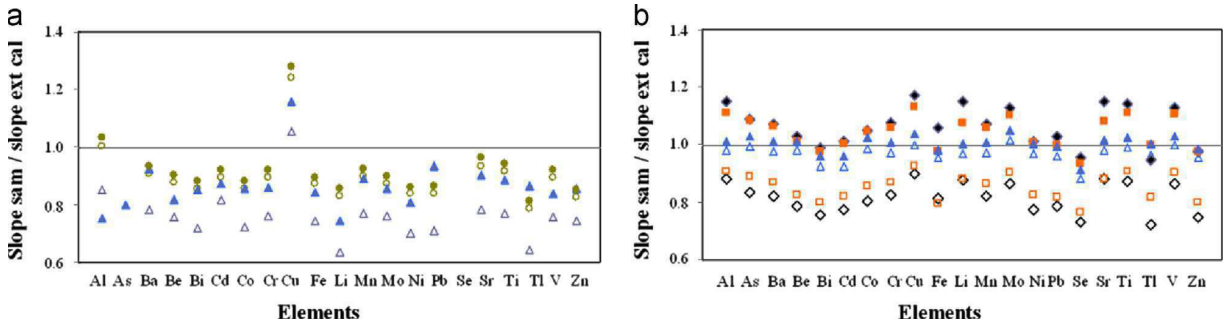

Fig. 1. (a) Ratio slope sample addition method calibration/slope external calibration when ultrasonic nebulizer is employed. Digested samples dilution 1/10 with (•) and without (○) internal standard; dilution 1/5 with (▲) and without (Δ) internal standard. (b) Ratio slope sample addition method calibration/slope external calibration when cross-flow nebulizer is employed. Digested samples dilution 1/5 with (▲) and without (Δ) internal standard; dilution 1/2 with (■) and without (□) internal standard; undiluted with (♦) and without (◇) internal standard.

Table 2
Effect of the sample dilution factor on the mineral concentration ($\mu g\ g^{-1}$ ww) of a pangafish using cross-flow nebulizer.

	Wavelength (nm)	Fresh sample								Liophilized sample					
		Undiluted		1/2 Dilution		1/5 Dilution		1/10 Dilution		Undiluted		1/2 Dilution		1/5 Dilution	
		With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS	With IS	Without IS
Al	309.271	nd	nd	2.1	nd	4.5	nd	nd	nd	21	18	28	21	45	32
	396.153	0.6	0.5	0.6	0.3	0.1	nd	nd	nd	4.1	5.2	3.8	1.8	4.9	nd
B	249.677	1.0	0.8	1.9	1.1	2.3	0.7	nd	nd	0.4	nd	nd	nd	nd	nd
	249.772	1.0	0.8	1.8	1.1	2.2	0.7	nd	nd	0.10	nd	nd	nd	nd	nd
Ba	233.527	0.10	0.04	nd	nd	nd	nd	nd	nd	nd	0.4	nd	0.5	nd	0.66
Ca	317.933	64	55	45	44	nd	nd	nd	nd	321	280	310	305	325	379
	396.847	66	40	40	10	nd	nd	nd	nd	347	292	297	298	214	321
Cr	267.716	0.08	nd	0.09	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cu	324.752	0.18	0.09	0.19	0.03	0.24	nd	0.07	nd	0.6	0.4	0.3	0.3	nd	nd
	327.393	0.14	0.07	0.16	0.02	0.18	nd	0.09	nd	0.3	0.6	nd	0.6	nd	0.7
Fe	238.204	2.1	1.7	2.1	1.7	1.0	0.8	1.7	1.1	22	19	22	18	16	17
	239.562	2.1	1.7	2.1	1.7	1.0	0.9	1.8	1.2	23	21	20	20	18	20
K	766.490	1581	1274	1563	1315	1414	1283	1449	1434	11524	9791	11037	10164	8545	10391
Mg	279.077	167	128	162	122	132	79	117	34	966	735	937	774	736	642
Mn	257.610	0.08	nd	0.01	nd	nd	nd	nd	nd	nd	0.4	nd	0.5	nd	0.5
	259.372	0.12	0.02	0.06	nd	nd	nd	nd	nd	0.12	0.5	nd	0.6	nd	0.8
Mo	202.031	0.04	0.04	0.04	0.03	0.06	nd	0.16	nd	nd	nd	nd	nd	nd	nd
	203.845	0.09	0.10	0.16	0.09	0.24	0.12	nd	nd	nd	nd	nd	nd	nd	nd
Na	330.237	6228	5114	6267	5360	5869	5359	6183	6066	16965	14166	16408	14766	16159	15291
	589.592	6089	5035	6175	5313	5746	5265	6146	6026	16460	13759	16189	14682	16039	15408
Sr	407.771	0.4	0.2	0.3	0.2	0.3	nd	0.2	nd	0.5	0.8	0.0	0.9	nd	1.1
	421.552	0.4	0.2	0.3	0.2	0.3	nd	0.2	nd	0.7	0.8	0.3	0.9	nd	1.3
V	290.880	0.05	nd	0.11	nd	0.25	nd	nd	nd	0.46	nd	0.39	nd	nd	0.11
	310.230	1.6	1.2	2.8	1.5	4.7	nd	nd	nd	15	10	19	13	34	28
Zn	206.200	1.8	1.5	1.8	1.5	1.6	1.2	1.3	0.8	14	10	14	11	13	11
	213.857	1.8	1.6	1.9	1.6	2.0	1.6	1.8	1.4	15	11	14	12	13	12

Not detected (nd) elements at any dilution: As, Be, Bi, Cd, Ce, Co, Dy, Er, Eu, Gd, Ho, La, Li, Lu, Nd, Ni, Pb, Pr, Sc, Se, Sm, Tb, Ti, Tl, Tm, Y, Yb.

being introduced into the plasma, the plasma thermal characteristics and the analyte excitation efficiency as well as the spatial distribution of the emitting species (Todolí et al., 2002).

In order to minimize the matrix effect, without increase the LODs, 0.5 g of sample was employed (double as the usually used 0.25 g). For that a pre-digestion step was necessary to avoid foam formation and overpressure during the digestion inside the microwave oven. On another hand, the volume of HNO_3 was reduced from 8 mL to 4 mL with the purpose of decreasing the rest of acid in digested samples and improving the nebulisation process. After digestion, the sample solution was transferred to a tube with the minimum volume of H_2O in order to minimize the dilution. This strategy allowed assaying different dilution factors later.

In general, under robust conditions i.e., high rf power, low carrier gas flow rate and large inner diameter of the injector, matrix effects can be minimized and the residual, depressive effect which could be observed in some cases has been assigned to aerosol generation and transport, thus indicating the crucial role of the nebulizer and spray chamber to obtain accurate results (Mermet, 1998).

As expected, in this work, the use of an internal standard is able to compensate partially the matrix effect in the main part of cases. This correction is more efficient in the case of the use of cross-flow nebulizer. So in spite of the high sensitivity obtained using ultrasonic nebulizer, in this case, cross- flow was preferred in order to reduce matrix effect. On the other hand, sample dilution is the simplest way to reduce matrix effects. As can be seen in Fig. 1 the dilution of sample decrease the matrix effect, being more comparable both slopes (standard addition method and external calibration).

From Table 2 (and Supplementary 1) we could again check that the use of an internal standard allows us to obtain comparable values for all dilutions in the main part of cases but it was observed that a high dilution factor has a detrimental effect on LOD and introduces mistakes in calculations of final concentrations. So the analysis of undiluted samples can be recommended.

Finally the use of lyophilized samples instead of fresh ones can provide additional advantages. Results shown in Table 2 (and Supplementary 2) lead to the conclusion that the measurement of undiluted samples with no matrix interferences is possible even if it does not suppose an advantage regarding the number of elements able to be determined in panga fish due to the low level of minerals in assayed samples.

Attending to spectra shape and baseline Er 349.910, K 404.721, Li 610.362, Sm 442.434, Tb 384.873, Tl 276.787 and V 310.230 were clearly rejected. Looking at the slopes of calibration lines (Supplementary 3) and by comparing results found at different dilution levels in preliminary studies, the spectral lines that were finally selected were: Al 396.153, As 188.979, B 249.772, Ba 233.527, Be 313.107, Bi 223.061, Ca 317.933, Cd 214.440, Ce 413.764, Co 228.616, Cr 267.716, Cu 324.752, Dy 353.170, Er 337.271, Eu 381.967, Fe 238.204, Gd 342.247, Ho 345.600, K 766.490, La 408.672, Li 670.784, Lu 261.542, Mg 279.077, Mn 257.610, Mo 203.845, Na 330.237, Nd 401.225, Ni 231.604, Pb 220.353, Pr 414.311, Sc 361.383, Se 196.026, Sm 359.260, Sr 407.771, Tb 350.917, Ti 336.121, Tl 190.801, Tm 313.126, V 290.880, Y 371.029, Yb 328.937, Zn 213.857.

Regarding analysis of certified sample (Table 4) in general, $\Delta_m \leq U_\Delta$, thus indicating that there was no significant difference between the measurement results and the certified values. Selenium bias is justified attending to small sensitivity in ICP-OES for this element (slope of calibration line 1893); whereas Cu and Sr were slightly affected by residual matrix effect.

4.2. Mineral content in panga fish samples

Results from this study are comparable with reported in the literature for panga fish. Major elements, Ca, K, Na and Mg were

Table 3
Analytical characteristics of ICP-OES determination of mineral elements in panga fish samples.

	Wavelenght (nm)	Calibration line	R^2	LOD[a] ($\mu g\ g^{-1}$)	LOQ[b] ($\mu g\ g^{-1}$)	Sam/extcal	RSD % ($n=10$)[c]	RSD % ($n=3$)[d]
Al	396.153	$I=120404C+471$	1.000	0.18	0.6	1.03	1.0	5–26 (0.7–1.4)
As	188.979	$I=1568C+0$	1.000	0.08	0.3	1.05	6.5	
B	249.772	$I=75901C+486$	1.000	0.014	0.05	1.03	1.4	6–20 (0.02–0.12)
Ba	233.527	$I=113871C+895$	1.000	0.04	0.4	1.01	1.4	2–29 (0.05–0.09)
Be	313.107	$I=1863670C+12125$	1.000	0.001	0.003	1.01	0.5	
Bi	223.061	$I=1970C-14$	0.999	0.10	0.3	1.04	5.0	
Ca	317.933	$I=13125C-6176$	1.000	0.5	1.5		3.0	1–10 (60–120)
Cd	214.440	$I=66146C+490$	1.000	0.002	0.008	0.99	1.3	
Ce	413.764	$I=104319C+565$	1.000	0.03	0.11			
Co	228.616	$I=27564C+365$	0.999	0.005	0.02	0.99	1.5	
Cr	267.716	$I=74903C+858$	1.000	0.2	0.8	1.03	1.8	
Cu	324.752	$I=334046C+4805$	1.000	0.007	0.02	1.08	1.3	3–19 (0.102–0.154)
Dy	353.170	$I=561856C+7$	1.000	0.0008	0.003			
Er	337.271	$I=305865C+36$	1.000	0.015	0.05			
Eu	381.967	$I=1339993C-294$	1.000	0.0004	0.0013			
Fe	238.204	$I=65029C+502$	1.000	1.6	5.5	0.99	0.3	3–20 (0.5–3.9)
Gd	342.247	$I=178427C+52$	1.000	0.004	0.014			
Ho	345.600	$I=384061C-135$	1.000	0.002	0.007			
K	766.490	$I=2149C-12622$	0.999	3	8.3		3.0	0.5–4 (100–2500)
La	408.672	$I=545285C-1018$	1.000	0.004	0.014			
Li	670.784	$I=97041C+804$	1.000	0.019	0.06	1.09	3.2	
Lu	261.542	$I=866666C-212$	1.000	0.0003	0.0008			
Mg	279.077	$I=1361C+34$	1.000	0.12	0.4		2.7	1–4 (150–200)
Mn	257.610	$I=407179C+5421$	1.000	0.006	0.02	1.01	0.4	2–15 (0.06–0.07)
Mo	203.845	$I=10349C+53$	1.000	0.014	0.05	1.07		
Na	330.237	$I=73C-5$	1.000	10	33.8		2.6	0.5–5 (3000–11000)
Nd	401.225	$I=133669C-122$	1.000	0.006	0.02			
Ni	231.604	$I=26496C+394$	1.000	0.03	0.10	0.99	2.1	
Pb	220.353	$I=5769C+17$	0.999	0.03	0.10	0.96	5.0	
Pr	414.311	$I=167236C+478$	1.000	0.016	0.05			
Se	196.026	$I=1893C+6$	1.000	0.08	0.25	1.05	13.3	
Sm	359.260	$I=99151C-83$	1.000	0.02	0.08			
Sr	407.771	$I=1982330C+19518$	1.000	0.005	0.02	1.08	2.7	0.3–6 (0.1–1.1)
Tb	350.917	$I=150421C+155$	1.000	0.003	0.011			
Ti	336.121	$I=443285C+3270$	1.000	0.011	0.04	1.04	0.5	
Tl	190.801	$I=1792C+16$	1.000	0.07	0.2	0.91	4.4	
Tm	313.126	$I=294340C+148$	1.000	0.006	0.02			
V	290.880	$I=91850C+942$	1.000	0.016	0.05	1.04	1.1	2–8 (0.08–0.15)
Y	371.029	$I=293779C+273$	1.000	0.003	0.010			
Yb	328.937	$I=2836077C-97$	1.000	0.0002	0.0008			
Zn	213.857	$I=81878C+1207$	1.000	0.04	0.14	0.97	1.3	1–8 (2.27–2.61)

Ext cal: slope external calibration; sam: slope standard addition method calibration.

[a] LODs were calculated as the concentration corresponding to signals equal to three-times the standard deviation of a blank solution and taking into consideration the amount of liophilized sample digested and the dilution after digestion as well as the relative moisture of the set of samples.

[b] LOQs were calculated as the concentration corresponding to signals equal to ten-times the standard deviation of a blank solution and taking into consideration the amount of liophilized sample digested and the dilution after digestion as well as the relative moisture of the set of samples.

[c] RSD data were calculated as a percentage from the average of the repeatibility of ten analysis made in 100 ng g^{-1} or 10 $\mu g\ g^{-1}$ (Ca, K, Mg, Na) standard solution.

[d] RSD data were calculated as a percentage from the average of the repeatibility of three independent analysis made in actual samples. The concentration ranges of those samples are indicated in brachets in $\mu g\ g^{-1}$.

found at averages levels of 90, 1700, 6000 and 170 $\mu g\ g^{-1}$, all of them at the same order than those reported previously (Orban et al., 2008) which were 80, 3400, 4000 and 120 $\mu g\ g^{-1}$, respectively. Mean values of Cu found varied in the range 0.10–0.15 $\mu g\ g^{-1}$ were lower than the reported 0.44 $\mu g\ g^{-1}$ (Adhikari et al., 2009) or 0.332 $\mu g\ g^{-1}$ (Mok et al., 2012); and Zn at an average level of 2.4 $\mu g\ g^{-1}$, lower than 3.240 $\mu g\ g^{-1}$ (Mok et al., 2012) but significantly higher than 0.5 $\mu g\ g^{-1}$ (Adhikari et al., 2009). Levels reported for Pb 0.024 and Cd 0.0026 $\mu g\ g^{-1}$(Szlinder-Richert et al., 2011) were difficult to compare because limits of detection in the employed method are 0.031 and 0.0024 $\mu g\ g^{-1}$, respectively. However Pb 0.14, Cd 0.10, Mn 0.11 $\mu g\ g^{-1}$ (Adhikari et al., 2009); and Pb 18.7, Cd 1.9 $\mu g\ g^{-1}$ dry weight (Phanwichien et al., 2010), were rather higher than those estimated in this study.

Additionally the element content profile obtained was compared with some data reported in the literature regarding other white fishes (Supplementary 4) (Guérin et al., 2011) concluding that mineral profile of panga fish is extremely close to spices like cod or hake and slightly differs in Fe, Sr and V with sole fish.

On the other hand, some selected elements were evaluated through their estimated daily intake (EDI), assuming consume of one 43.3 g fish portion per day (AESAN, 2011b), and by comparing them with the recommended values (RDI) for adults 31–50 years old (Institute of Medicine, 2011) (Table 5). It should be noticed the high amount of Na that represents around 20 percent of RDI and could be explained, as have referred Orban et al. and Esposito et al., by the additivation with E-451 (sodium tripolyphosphate), known to increase the durability of the product. The content of Mg and K is also considerable because it supposes around 2 percent of RDI. Other essential elements such as Fe, Zn and Cu are present in amount that cause around 1 percent RDI in contrast with the minimum amount of Mn, Sr, V or the absence of Co, Mo, Se which are considered beneficial as well.

Table 4
Results obtained through the analysis of a certified sample TORT-2 (lobster hepatopancreas).

	C_m[a] ($\mu g\ g^{-1}$)	C_{CRM}[b] ($\mu g\ g^{-1}$)	Accuracy (%)[c]	Statistical analysis		
				Δ_m[d]	U_Δ[e]	
As	23.9 ± 0.9	21.6 ± 1.8	89 ± 4	2.3	2.3	Comparable
Cd	27 ± 2	26.7 ± 0.6	99 ± 7	0.3	3.1	Comparable
Co	nd	0.51 ± 0.09		0.51	2.00	Comparable
Cr	1.00 ± 0.17	0.77 ± 0.15	70 ± 20	0.23	2.01	Comparable
Cu	142 ± 11	106 ± 10	66 ± 10	36	13	Not comparable
Fe	110 ± 14	105 ± 13	95 ± 13	5	16	Comparable
Mn	14.6 ± 1.2	13.6 ± 1.2	93 ± 9	1	2	Comparable
Mo	1.3 ± 0.3			0.35	2.03	Comparable
Ni	2.6 ± 0.3	2.50 ± 0.19	96 ± 12	0.1	2.0	Comparable
Pb	nd			0.35	2.00	Comparable
Se	0.84 ± 0.45	5.6 ± 0.7	15 ± 8	4.76	2.07	Not comparable
Sr	59 ± 5	45.2 ± 1.9	69 ± 11	13.8	6.1	Comparable
V	2.7 ± 0.8	1.64 ± 0.19	40 ± 50	1.06	2.20	Comparable
Zn	187 ± 15	180 ± 6	96 ± 8	7	17	Comparable
Hg	0.280 ± 0.057	0.270 ± 0.060	100 ± 20	0.010	65.850	Comparable

[a] Mean measured value corresponding to three independent analysis.
[b] Certified value.
[c] Degree of accuracy, calculated as: $100-|C_m-C_{CRM}|/C_{CRM}{}^* 100|$.
[d] Absolute difference between mean measured value and certified value.
[e] Expanded uncertainty of difference between result and certified value, calculated as $U_\Delta=2^*(sd/n^{1/2}+U_{CRM}/2)^{1/2}$. Sd: standard deviation, n: number of replicates, U_{CRM}: Expanded uncertainty of each certified value given on the certificate.

Table 5
Mineral profile of panga fish samples. Concentration expressed in $\mu g\ g^{-1}$ fw (average ± sd, three replicate analysis)[a].

	S1	S2	S3	S4	S5	S6	S7	Mean ± sd (7 individuals)	% RDI[b]	% TDI[c]
Al	1.4 ± 0.3	1.35 ± 0.13	1.19 ± 0.09	1.13 ± 0.06	1.0 ± 0.3	1.2 ± 0.2	0.71 ± 0.10	1.1 ± 0.2		0.2–0.3
B	0.021 ± 0.004	0.12 ± 0.02	0.111 ± 0.013	0.071 ± 0.005	0.053 ± 0.011	nd	0.024 ± 0.002	< 0.07		
Ba	nd	0.053 ± 0.006	0.08 ± 0.02	0.086 ± 0.002	nd	nd	nd	< 0.07		
Ca	81 ± 8	120 ± 4	110.3 ± 1.0	88 ± 3	76 ± 3	65.0 ± 1.3	55 ± 2	80 ± 20	0.3–0.4	
Cu	0.11 ± 0.02	0.122 ± 0.003	0.154 ± 0.006	0.139 ± 0.006	0.15 ± 0.02	0.143 ± 0.007	0.102 ± 0.008	0.131 ± 0.019	0.5–0.7	0.014–0.19
Fe	0.51 ± 0.08	0.75 ± 0.08	1.87 ± 0.15	0.94 ± 0.02	1.9 ± 0.3	1.33 ± 0.10	3.9 ± 0.8	1.6 ± 1.1	0.3–1.5	0.04–0.21
K	1900 ± 100	1010 ± 40	1074 ± 5	1960 ± 30	2490 ± 70	2210 ± 40	1990 ± 50	1800 ± 600	1.1–2.2	
Mg	170 ± 8	153 ± 3	150 ± 2	194 ± 3	197 ± 7	180 ± 4	167 ± 4	173 ± 19	1.6–2.0	
Mn	nd	nd	nd	0.070 ± 0.002	0.065 ± 0.010	nd	nd	< 0.068	0–0.14	
Na	5700 ± 300	11300 ± 200	10760 ± 50	7190 ± 70	2600 ± 100	3350 ± 80	2930 ± 70	6000 ± 4000	5.8–29	
Sr	0.206 ± 0.005	1.086 ± 0.003	0.971 ± 0.012	0.350 ± 0.002	0.238 ± 0.012	0.103 ± 0.006	0.090 ± 0.005	0.4 ± 0.4		
V	0.112 ± 0.009	0.146 ± 0.010	0.131 ± 0.003	0.116 ± 0.008	0.087 ± 0.007	0.094 ± 0.002	0.080 ± 0.004	0.11 ± 0.02		
Zn	2.27 ± 0.11	2.44 ± 0.04	2.48 ± 0.02	2.31 ± 0.02	2.44 ± 0.12	2.61 ± 0.03	2.5 ± 0.2	2.44 ± 0.12	0.9–1.0	0.14–0.54
Hg (10^3)	2.15 ± 0.05	1.52 ± 0.12	2.11 ± 0.12	3.9 ± 0.3	1.565 ± 0.004	1.552 ± 0.012	2.54 ± 0.03	2.03 ± 0.05		0.031–0.032

[a] Not detected elements (nd) in any sample: As, Be, Bi, Cd, Ce, Co, Cr, Dy, Er, Eu, Gd, Ho, La, Li, Lu, Mo, Nd, Ni, Pb, Pr, Sc, Se, Sm, Tb, Ti, Tl, Tm, Y, Yb.
[b] % RDI = EDI/RDI * 100. EDI: estimated daily intake calculated from mean data results and taking into account a diary consume of 43.3 g (AESAN, 2011b) RDI: recommended daily intake (man 31–50 years old), in $mg\ d^{-1}$: Ca 1000, Cu 0.9, Fe 8, K 4700, Mg 420, Mn 2.3, Na 1500, Zn 11 (Institute of Medicine, 2011).
[c] % TDI = EI/TDI * 100. TDI: tolerable daily intake calculated for 70 kg body weight from reported PTWI values, in $mg\ kg^{-1}\ bw^{-1}$: Al 2; and PMTDI values, in $mg\ kg^{-1}\ bw^{-1}$: Cu 0.05–0.5, Fe 0.8, Hg 0.004, Zn 0.3–1 (JECFA, 1982, 1983, 2011a, 2011b).

As can be also seen in Table 5, EDI Al, Cu, Fe and Zn of a 70 kg adult human, were compared with provisional tolerable weekly intake (PTWI) or provisional maximum tolerable daily intake (PMTDI) established by the FAO/WHO Expert committee on Food Additives (JECFA, 1982, 1983, 2011b, 2011a), concluding that all considered elements were under tolerable values. Finally, it should be noticed that the maximum limits recommended by European Commission for Cd (0.05 $mg\ kg^{-1}$) and Pb (0.3 $mg\ kg^{-1}$) were not exceeded in any analysed sample (EEC, 2006, 2011).

4.3. Mercury concentration in panga fish

Reported levels of Hg varied between 0.41 (Ferrantelli et al., 2012) 0.3 (Orban et al., 2008), 0.16 (Pal et al., 2011), 0.099 (Mok et al., 2012) and 0.005 $\mu g\ g^{-1}$ (Szlinder-Richert et al., 2011) and were mildly higher that those found in this study.

Considering EDI and PTWI value for Hg (Table 5) the maximum limit recommended by European Commission (0.5 $mg\ kg^{-1}$) safety for human consumption can be assessed.

5. Conclusions

The methodology developed to determine major and trace elements in panga fish by ICP-OES after microwave assisted acid digestion, and the determination by atomic absorption after ashing of fresh samples have been successfully applied to the determination of the mineral profile of Vietnam panga fish samples. Data obtained clearly indicate a low mineral profile for this kind of products and the absence of toxic elements. Although this methodology should be applied to a large number of samples in order to obtain statistically significant information. However, results obtained provided evidences that suggest the absence of health risks associated with panga intake in clear contrast with informations available on internet

(OCU, 2010; Fish Information & Services, 2010; Greenpeace, 2012) which create a social alert about the consume of this fish.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2013.06.003.

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Supplementary 1

Effect of the dilution factor on the mineral concentration (µg g^{-1}) found in fresh panga fish samples when cross-flow nebulizer is employed

Element	Wavelength (nm)	undiluted						1/2 dilution					
		S1		S2		S3		S1		S2		S3	
		with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS
Al	396.153	0.7	0.5	0.8	0.5	0.6	0.5	0.9	0.5	0.7	0.5	0.9	0.5
B	249.677	0.5	0.4	1.0	0.6	1.0	0.8	1.4	0.6	1.2	0.8	2.1	1.1
	249.772	0.5	0.3	1.0	0.6	1.0	0.8	1.4	0.6	1.2	0.8	2.1	1.1
Ba	233.527	nd	nd	0.03	nd		nd	nd	nd	nd	nd	nd	nd
	493.408	nd	nd	0.06	nd	0.04	nd	nd	nd	nd	nd	nd	nd
Ca	317.933	39	34	80	65	70	60	25	26	59	59	58	53
	396.847	39	17	82	50	73	46	17	nd	55	26	54	19
Cr	267.716	0.03	nd	0.05	nd		nd	0.11	nd	0.09	nd	0.09	nd
Cu	324.752	0.14	0.06	0.12	0.03	0.10	0.03	0.20	0.03	0.11	nd	0.16	nd
	327.393	0.12	0.04	0.10	0.02	0.08	nd	0.16	0.00	0.11	nd	0.12	nd
Fe	238.204	nd	nd	nd	nd	nd	nd	nd	nd	2.19	nd	nd	nd
	239.562	nd	nd	nd	nd	nd	nd	nd	nd	2.22	nd	nd	nd
K	404.721	1395	1129	581	449	675	553	1215	964	568	464	710	532
	766.490	1413	1130	584	456	765	621	1451	1201	561	493	777	640
Mg	279.077	124	92	98	68	116	86	126	89	91	64	118	81
	285.213	116	91	88	66	107	85	108	85	73	61	99	77
Mn	257.610	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	259.372	0.03	nd	nd	nd	0.02	nd	nd	nd	nd	nd	nd	nd
Mo	202.031	0.46	0.39	0.13	0.11	0.08	0.07	0.82	0.69	0.13	0.12	0.12	0.09
	203.845	0.52	0.45	0.19	0.16	0.08	0.09	0.92	0.74	0.25	0.20	0.24	0.14
Na	330.237	5531	4509	7770	6168	8755	7230	5850	4922	7576	6723	9193	7671
	589.592	5458	4480	7525	6015	8397	6982	5701	4826	7397	6603	8949	7516
Sr	407.771	0.2	0.1	0.8	0.6	0.8	0.6	0.2	0.1	0.8	0.6	0.8	0.5
	421.552	0.2	0.1	0.8	0.6	0.8	0.6	0.2	0.1	0.8	0.6	0.8	0.5
V	290.880	0.05	nd	0.06	nd	0.05	nd	0.17	nd	0.06	nd	0.18	nd
Zn	206.200	1.7	1.4	1.5	1.2	1.7	1.4	1.8	1.4	1.6	1.3	1.8	1.4
	213.857	1.7	1.5	1.6	1.3	1.7	1.5	1.9	1.6	1.6	1.4	2.0	1.6

Supplementary 1 - Continuation

Element	Wavelength (nm)	1/5 dilution						1/10 dilution					
		S1		S2		S3		S1		S2		S3	
		with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS
Al	396.153	0.9	0.6	0.8	0.5	0.6	0.3	0.2	0.1	0.1	0.1	1.2	0.9
B	249.677	1.2	nd	1.7	nd	2.3	0.3	1.6	nd	nd	nd	1.3	nd
	249.772	1.1	nd	1.6	nd	2.2	0.4	1.7	nd	nd	nd	1.5	nd
Ba	233.527	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	493.408	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ca	317.933	nd	nd	4	22	nd	18	nd	nd	nd	nd	nd	nd
	396.847	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cr	267.716	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cu	324.752	0.24	nd	0.22	nd	0.21	nd	0.17	nd	0.11	nd	0.20	nd
	327.393	0.18	nd	0.16	nd	0.18	nd	0.13	nd	0.05	nd	0.15	nd
Fe	238.204	nd	nd	2.39	nd	nd	nd	nd	nd	nd	nd	nd	nd
	239.562	nd	nd	2.47	nd	nd	nd	nd	nd	nd	nd	nd	nd
K	404.721	1700	1434	523	387	882	702	1538	1530	nd	nd	nd	nd
	766.490	1478	1315	554	505	725	653	1364	1330	465	486	675	657
Mg	279.077	114	60	80	29	98	45	80	nd	48	nd	73	nd
	285.213	64	49	29	18	46	33	nd	nd	nd	nd	nd	nd
Mn	257.610	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	259.372	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Mo	202.031	0.57	0.44	0.21	0.11	0.16	nd	1.80	1.57	0.65	0.46	0.29	nd
	203.845	0.44	0.27	0.18	nd	0.30	0.14	1.65	1.64	nd	nd	0.18	nd
Na	330.237	6065	5437	8070	7273	8957	8032	5835	5631	7770	7583	9157	8584
	589.592	5991	5392	7928	7178	8842	7968	5764	5559	7631	7454	9045	8500
Sr	407.771	0.2	nd	0.7	0.4	0.7	0.4	0.1	nd	0.6	0.1	0.6	0.0
	421.552	0.2	nd	0.7	0.4	0.7	0.4	0.1	nd	0.6	0.1	0.6	0.1
V	290.880	0.27	nd	0.27	nd	0.26	nd	0.06	nd	0.04	nd	0.26	nd
Zn	206.200	1.6	1.1	1.4	1.0	1.7	1.3	1.3	0.7	1.2	0.6	1.4	0.8
	213.857	2.0	1.6	1.8	1.5	2.1	1.7	1.9	1.4	1.7	1.2	2.1	1.5

Supplementary 2

Effect of the dilution factor on the mineral concentration (μg g^{-1}) found in liophilizated panga fish samples when cross flow nebulizer is employed

Element	Wavelength (nm)	undiluted											
		S1		S2		S3		S4		S5		S6	
		with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS
Al	396.153	7.8	6.7	7.8	6.7	6.9	6.1	6.5	6.4	5.6	6.3	7.2	7.2
B	249.677	1.1	0.2	1.1	0.2	1.0	0.1	0.8	0.0	0.7	nd	0.4	nd
	249.772	0.7	0.2	0.7	0.2	0.6	0.2	0.4	0.0	0.3	nd		nd
Ba	233.527	0.31	0.63	0.31	0.63	1.13	0.73	0.50	0.78	nd	0.49	nd	
	493.408	0.38	nd	0.38	nd	1.25	nd	0.59	nd	nd	nd	nd	nd
Ca	317.933	698	532	698	532	640	490	513	416	441	376	377	320
	396.847	776	566	776	566	707	518	570	443	486	399	412	337
Cu	324.752	0.70	0.38	0.70	0.38	0.89	0.50	0.81	0.51	0.84	0.59	0.83	0.57
	327.393	0.29	0.54	0.29	0.54	0.51	0.67	0.45	0.67	0.55	0.77	0.53	0.74
Fe	238.204	4.37	3.83	4.37	3.83	10.85	8.14	5.47	4.88	11.13	9.26	7.73	6.65
	239.562	3.66	4.63	3.66	4.63	10.47	9.16	4.83	5.73	10.78	10.33	7.01	7.45
K	766.490	5774	4437	5774	4437	6228	4792	11392	9245	14442	12252	12229	10239
Mg	279.077	886	613	886	613	868	600	1123	817	1145	870	1042	782
Mn	257.610	nd	0.35	nd	0.35	0.03	0.39	0.41	0.69	0.38	0.69	nd	0.37
	259.372	0.18	0.50	0.18	0.50	0.27	0.55	0.65	0.84	0.57	0.82	0.10	0.47
Na	330.237	65303	49547	65303	49547	62392	47367	41674	33232	14621	12182	19404	15982
	589.592		nd		nd		nd	40407	32223	14540	12129	19057	15692
Sr	407.771	6.3	4.5	6.3	4.5	5.6	4.0	2.0	1.8	1.4	1.4	0.6	0.9
	421.552	6.0	4.2	6.0	4.2	5.5	3.8	2.0	1.7	1.5	1.4	0.7	0.9
V	290.880	0.85	nd	0.85	nd	0.76	nd	0.67	nd	0.50	nd	0.55	nd
Zn	206.200	13.6	8.9	13.6	8.9	13.8	9.0	13.0	8.9	13.9	10.0	15.0	10.7
	213.857	14.1	9.1	14.1	9.1	14.4	9.3	13.4	9.1	14.2	10.1	15.1	10.7

Supplementary 2 - Continuation

Element	Wavelength (nm)	1/2 dilution											
		S1		S2		S3		S4		S5		S6	
		with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS
Al	396.153	6.3	3.2	7.8	3.6	6.4	2.3	6.6	2.7	5.2	2.4	8.4	4.6
B	249.677	nd	nd	0.7	nd	0.8	nd	0.7	nd	0.4	nd	nd	nd
	249.772	nd	nd	0.1	nd	0.2	nd	nd	nd	nd	nd	nd	nd
Ba	233.527	nd	0.31	nd	0.75		0.85	nd	0.89	nd	0.58	nd	0.20
	493.408	nd	nd	nd	nd	0.83	nd	nd	nd	nd	nd	nd	nd
Ca	317.933	411	377	668	564	611	506	508	434	416	386	403	366
	396.847	412	376	707	579	643	517	530	443	428	393	407	367
Cu	324.752	0.33	0.28	0.42	0.27	0.69	0.40	0.61	0.38	0.55	0.48	0.60	0.46
	327.393	nd	0.59	nd	0.59	0.09	0.71		0.68	0.11	0.79	0.14	0.79
Fe	238.204	2.29	2.72	3.85	3.52	11.27	8.22	5.27	4.49	10.91	8.43	7.77	6.65
	239.562	nd	3.67	1.55	4.59	9.50	9.68	3.07	5.63	7.87	9.76	5.71	7.86
K	766.490	10525	9255	5552	4597	6006	4849	11315	9367	14107	12568	12231	10623
Mg	279.077	955	753	881	658	863	629	1136	844	1124	897	1054	821
Mn	257.610	nd	0.45	nd	0.40	nd	0.44	nd	0.75	nd	0.76	nd	0.42
	259.372	nd	0.60	nd	0.60	nd	0.65	0.31	0.95	0.20	0.93	nd	0.57
Na	330.237	31716	27343	64520	52887	61688	49295	41832	34017	14305	12447	19513	16589
	589.592	31322	27121	62730	51481	60132	48105	41464	33795	14401	12621	19417	16608
Sr	407.771	0.6	1.3	5.6	4.7	5.0	4.2	1.5	1.9	0.8	1.5	0.1	1.0
	421.552	1.0	1.4	6.0	4.9	5.4	4.3	1.8	1.9	1.1	1.5	0.4	1.0
V	290.880	0.60	nd	0.90	0.05	1.00	0.02	0.98	nd	0.55	nd	0.67	nd
Zn	206.200	12.5	9.4	13.4	9.5	14.0	9.7	13.5	9.5	14.2	10.8	15.6	11.6
	213.857	12.9	9.8	14.1	10.1	14.7	10.2	14.0	9.9	14.5	11.1	15.9	11.8

Supplementary 2 - Continuation

Element	Wavelength (nm)	1/5dilution											
		S1		S2		S3		S4		S5		S6	
		with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS	with IS	without IS
Al	396.153	6.8	nd	6.1	nd	5.5	nd	5.3	nd	4.4	nd	6.4	nd
B	249.677	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	249.772	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ba	233.527	nd	0.53	nd	0.97	nd	1.57	nd	1.14	nd	0.80	nd	0.81
	493.408	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Ca	317.933	488	522	610	619	564	581	456	491	394	442	352	407
	396.847	411	485	545	587	492	545	370	448	298	394	246	353
Cu	324.752	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	327.393	nd	0.75	nd	0.68	nd	0.85	nd	0.81	nd	0.91	nd	0.90
Fe	238.204	nd	nd	1.18	1.52	6.46	6.74	2.18	2.76	7.01	7.98	3.80	8.34
	239.562	nd	nd	2.31	3.35	7.89	8.91	3.33	4.55	7.35	10.03	5.02	10.40
K	766.490	7871	7879	3793	4348	4042	4672	8086	9591	10559	12841	8689	10713
Mg	279.077	743	646	661	551	635	532	844	720	868	756	764	676
Mn	257.610	nd	0.60	nd	0.54	nd	0.58	nd	0.91	nd	0.91	nd	0.53
	259.372	nd	0.91	nd	0.88	nd	0.92	nd	1.24	nd	1.21	nd	0.80
Na	330.237	39426	36963	60840	55674	56663	52243	39159	36577	13817	13083	17765	17131
	589.592	39444	37177	60334	55440	56144	51854	39081	36670	13893	13311	17700	17222
Sr	407.771	nd	1.6	3.9	5.1	3.2	4.6	0.1	2.2	nd	1.8	nd	1.2
	421.552	nd	1.8	3.8	5.3	3.2	4.8	0.0	2.4	nd	1.9	nd	1.3
V	290.880	nd	nd	nd	0.15	nd	0.14	nd	0.10	nd	nd	nd	0.08
Zn	206.200	10.8	9.3	10.9	9.1	11.2	9.4	10.7	9.2	12.1	10.5	12.8	11.3
	213.857	11.4	10.5	11.8	10.4	11.9	10.6	11.3	10.4	12.5	11.6	13.1	12.4

Supplementary 3

ICP-OES sensitivity at different spectral lines

	Wavelenght (nm)	Calibration line	R^2	LOD (µg mL^{-1})
Al	309.271	$I = 86529C+5483$	0.999	0.07
	396.153	$I = 120404C+471$	1.000	0.03
As	188.979	$I = 1568C+0$	1.000	0.011
	193.696	$I = 1426C+26$	1.000	0.013
B	249.677	$I = 38633C + 318$	1.000	0.0015
	249.772	$I = 75901C + 486$	1.000	0.0020
Ba	233.527	$I = 113871C + 895$	1.000	0.006
	493.408	$I = 3500427C + 39280$	1.000	0.006
Be	313.042	$I = 3730568C + 52992$	1.000	0.0010
	313.107	$I = 1863670C + 12125$	1.000	0.0002
Bi	223.061	$I = 1970C - 14$	0.999	0.014
	306.766	$I = 22790C +2704$	0.999	0.196
Ca	317.933	$I = 13125C -6176$	1.000	0.07
	396.847	$I = 664524C + 550080$	1.000	0.08
Cd	214.440	$I = 66146C + 490$	1.000	0.0003
	228.802	$I = 4478C + 575$	1.000	0.0006
Ce	413.764	$I = 104319C +565$	1.000	0.005
Co	228.616	$I = 27564C + 365$	0.999	0.0008
	238.892	$I = 85385C + 956$	1.000	0.0010
Cr	267.716	$I = 74903C + 858$	1.000	0.03
Cu	324.752	$I = 334046C + 4805$	1.000	0.0010
	327.393	$I = 207283C + 2082$	1.000	0.0010
Dy	353.170	$I = 561856C + 7$	1.000	0.0001
Er	337.271	$I = 305865C + 36$	1.000	0.002
	349.910*	$I = 233983C + 41$	1.000	0.002
Eu	381.967	$I = 1339993C -294$	1.000	0.0001
	412.970	$I = 1592314C + 144$	1.000	0.0002
Fe	238.204	$I = 65029C + 502$	1.000	0.2
	239.562	$I = 89031C + 567$	1.000	0.2
Gd	336.223	$I = 86846C - 377$	1.000	0.0008
	342.247	$I = 178427C + 52$	1.000	0.0006
Ho	339.898	$I = 148907C + 132$	1.000	0.0013
	345.600	$I = 384061C - 135$	1.000	0.0003
K	404.721*	$I = 9C + 58$	0.990	12.9
	766.490	$I = 2149C - 12622$	0.999	0.4
La	379.478	$I = 307165C -543$	1.000	0.0004
	408.672	$I = 545285C -1018$	1.000	0.0006
Li	610.362*	$I = 10607C + 111$	1.000	0.012
	670.784	$I = 97041C + 804$	1.000	0.003
Lu	261.542	$I = 866666C -212$	1.000	0.00004
	291.139	$I = 146827C -99$	1.000	0.00037
Mg	279.077	$I = 1361C + 34$	1.000	0.018
	285.213	$I = 144C -88$	1.000	5.605
Mn	257.610	$I = 407179C + 5421$	1.000	0.0009
	259.372	$I = 603816C + 6647$	1.000	0.0012
Mo	202.031	$I = 22640C + 203$	1.000	0.0010
	203.845	$I = 10349C + 53$	1.000	0.0021
Na	330.237	$I = 73C -5$	1.000	1.5
	589.592	$I = 15622C -7565$	1.000	1.1

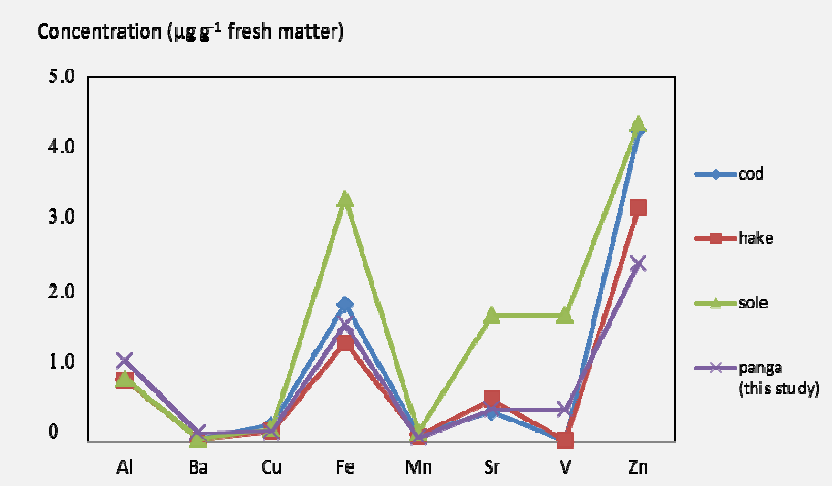
Supplementary 3 - Continuation

	Wavelenght (nm)	Calibration line	R^2	LOD[a] (µg mL^{-1})
Nd	401.225	$I = 133669C - 122$	1.000	0.0009
	406.109	$I = 145178C + 72$	1.000	0.0020
Ni	231.604	$I = 26496C + 394$	1.000	0.004
	232.003	$I = 17632C + 182$	1.000	0.005
Pb	217.000	$I = 1910C + 22$	1.000	0.020
	220.353	$I = 5769C + 17$	0.999	0.005
Pr	390.844	$I = 118577C - 642$	1.000	0.0014
	414.311	$I = 167236C + 478$	1.000	0.0024
Se	196.026	$I = 1893C + 6$	1.000	0.011
	203.985	$I = 976C - 13$	0.996	0.017
Sm	359.260	$I = 99151C - 83$	1.000	0.003
	442.434*	$I = 107450C + 1338$	1.000	0.006
Sr	407.771	$I = 1982330C + 19518$	1.000	0.0007
	421.552	$I = 915234C + 8537$	1.000	0.0007
Tb	350.917	$I = 150421C + 155$	1.000	0.0005
	384.873*	$I = 195118C + 108$	1.000	0.0012
Ti	334.940	$I = 466614C + 4351$	1.000	0.0013
	336.121	$I = 443285C + 3270$	1.000	0.0016
Tl	190.801	$I = 1792C + 16$	1.000	0.009
	276,787*	$I = 3106C + 33$	1.000	0.008
Tm	313.126	$I = 294340C + 148$	1.000	0.0009
	346.220	$I = 207182C + 24$	1.000	0.0002
V	290.880	$I = 91850C + 942$	1.000	0.002
	310.230*	$I = 294719C + 12117$	1.000	0.039
Y	324.227	$I = 135804C - 99$	1.000	0.0010
	371.029	$I = 293779C + 273$	1.000	0.0004
Yb	328.937	$I = 2836077C - 97$	1.000	0.00004
	369.419	$I = 1046696C - 8$	1.000	0.00005
Zn	206.200	$I = 23082C + 204$	1.000	0.006
	213.857	$I = 81878C + 1207$	1.000	0.006

LODs were calculated as the concentration corresponding to signals equal to three-times the standard deviation of a blank solution.
(*) Interfered spectra

Supplementary 4

Comparison with “white fish” mineral profiles available from the literature (Guérin et al., 2011).



1.4. Estudio comparativo de los procedimientos de preparación de muestra para análisis de suplementos alimenticios

A comparative study on sample preparation procedures for supplementary foods by ICP-OES: Green chemistry considerations

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A comparative study on sample preparation procedures for supplementary foods by ICP-OES: Green chemistry considerations†

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An inductively coupled plasma optical emission (ICP-OES) method was developed for the simultaneous determination of major, minor and trace elements in food. Preliminary studies focused on selecting suitable operational conditions in order to provide the highest sensitivity and to maximize the number of analytes to be determined without spectral or matrix interference. Ruthenium and rhenium were evaluated as internal standards and samples were analyzed at different dilution levels. Furthermore, a comparative study was carried out by using three sample digestion methods, microwave-assisted digestion with HNO_3/H_2O_2, dry ashing and dry ashing with $Mg(NO_3)_2/MgO$ as an ashing aid. Adequate precision and accuracy were assured by the analysis of spiked samples and certified reference material NIST 1573a tomato leaves. The method was successfully applied to the analysis of supplementary foods.

1. Introduction

Inductively coupled plasma optical emission spectroscopy (ICP-OES) is an attractive technique for mineral analysis in most laboratories because it offers the possibility to obtain quantitative data, over wide concentration ranges for several elements with low consumption of samples, reagents and time. However, accurate results require selecting appropriate procedures for each type of sample, including ICP-OES instrumental conditions and suitable sample treatment.

The high working temperature of plasma causes spectral interference that may be overcome by suitable emission line selection. Furthermore, the matrix composition, specially organic matter, inorganic acids and easily ionized elements, such as alkali metals, could strongly disturb the analytical signals in ICP OES, thus affecting the aerosols generated by the nebulizer, the chemical state in which the target analytes are introduced into the plasma, the plasma thermal characteristics, the analyte excitation efficiency and the spatial distribution of the emitting species.[1] To solve these problems several strategies such as sample dilution, matrix separation, matrix matching, standard addition calibration or internal standardization have been proposed in the literature.[2]

Metal and metalloid ordinary determination by ICP-OES requires preliminary treatment of samples to move from solids to homogenous liquid solutions.[3] Numerous studies focus on comparing the applicability of the most commonly used digestion procedures, such as dry ashing and wet digestion, over different matrices.[4–9] Dry ashing has the capability of handling large samples, ensures complete elimination of the organic matter and provides a high pre-concentration factor. The main drawbacks are associated with losses of volatile compounds but it can be overcome by using ashing aids such as $Mg(NO_3)_2$–MgO.[10] Wet digestion is prone to provide problems with insoluble compounds or coprecipitation but the use of microwave assisted procedures has minimized these troubles. Microwave assisted digestion has been demonstrated to be a clean, simple and rapid sample digestion procedure. However the use of strong acids can contribute to increase the matrix effects.

In foodstuff analysis, ICP-OES has gradually assumed prime importance because of the growing demand of information about mineral composition, including harmful and essential elements. Furthermore new lifestyles involve new feed habits and also the occurrence of new food products that should be analysed to assess their nutritional and/or hazardous properties.

The aim of the present study has been the development and validation of a simple, sensitive and fast analytical procedure for determining the mineral profile of supplementary foods, including a comparison between dry ashing and microwave digestion for sample pre-treatment dissolution and the establishment of suitable conditions for ICP-OES analysis.

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Analytical Methods Technical Note

2. Experimental

2.1. Instruments and apparatus

ICP-OES measurements were performed using a dual-viewed spectrometer Perkin Elmer model Optima 5300 DV (Norwalk, CT, USA) with an auto sampler A-93-plus, cross-flow nebulizer and Scott spray chamber.

Dry mineralization was performed using a muffle furnace for the simultaneous treatment of 40 samples, equipped with a Eurotherm 2416 controller Biometa (Asturias, Spain). A microwave labstation Ethos SEL from Milestone (Sorisole, Italy), equipped with a thermocouple sensor for automatic temperature control and automatic gas leaks detector, was employed for the simultaneous microwave-assisted digestion of 10 samples introduced inside high pressure vessels of 100 mL inner volume. For cleaning the vessels with sub-boiling HNO_3 an automatic cleaning device Trace-Clean from Milestone was used.

Other equipment included a sand bath and an ultrasound water bath of 9 L volume with 50 W power and 50 Hz frequency, both from Selecta (Barcelona, Spain).

Ultrapure water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q system Millipore (Bedford, USA).

2.2. Material, reagents and standards

All chemicals used were of the highest purity available and all solutions were prepared in ultrapure water. For sample preparation HCl 37% (w/v) from Merck (Darmstadt, Germany), HNO_3 69% (w/v), H_2O_2 35% (w/v), $Mg(NO_3)_2 \cdot 6H_2O$ and MgO, all from Scharlau (Barcelona, Spain) were employed. Argon C-45 (purity higher than 99.995%) used as the plasmogen and carrier gas in the ICP-OES system was supplied by Carburos Metálicos (Barcelona, Spain).

Table 1 ICP-OES instrumental operating conditions employed through this study

Type of detector	Solid state
Type of nebulizer	Cross-flow
Type of spray chamber	Scott
Injector tube diameter (mm)	0.3
RF generator power (W)	1300 W
Frequency of RF generator (MHz)	40.68
Plasma argon flow rate (L min^{-1})	15
Auxiliary argon flow rate (L min^{-1})	0.2
Nebulization gas flow rate (L min^{-1})	0.8
Sample aspiration rate (mL min^{-1})	1.1
Torch configuration	Axial (radial for Ca, K, Mg, Na, and Sr)
Integration and reading time (sec)	10
Background correction	2-points
Replicate number	3

Calibration standards were prepared by diluting stock standard solutions from Scharlau. For minor and trace elements, a multielement solution of Al, As, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Se, Sr, Ti, Tl, V, and Zn containing 100 mg L^{-1} of each element in 5% HNO_3 (v/v) was employed. Additionally, a multielement solution of Ce, La, Nd and Pr 100 mg L^{-1} and Dy, Er, Eu, Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb and Y 20 mg L^{-1} in 5% (v/v) HNO_3 and Sn 1000 mg L^{-1} in 0.5 M HNO_3 was employed. Major element Ca, K, Mg, and Na standards were prepared from the corresponding monoelemental standards of 1000 mg L^{-1} in 0.5 M HNO_3. A Ru solution of 1000 mg L^{-1}, prepared in 1 M HCl, and a Re solution of 100 mg L^{-1}, prepared in 0.5 M HNO_3, both from Fluka (Buchs, Switzerland) were used as internal standards (ISs).

All the plastic and glassware materials were cleaned by soaking them in HNO_3 10% (v/v) for 24 h, rinsed with ultrapure water three times and dried at room temperature before use.

2.3. Commercial and reference samples

Supplementary foods mainly composed of crushed cereals were acquired in health food stores in Brazil.

NIST 1573a (tomato leaves) was used as a vegetable certified reference material for method development and validation and also in the analysis of every run of samples.

2.4. Pre-treatment of samples

2.4.1. Microwave acid digestion procedure. The microwave-assisted treatment was adapted from that employed by us for the determination of the mineral profile of diets.[11]

Samples were accurately weighed (0.5 g) inside dry and clean Teflon vessels. Eight mL of concentrated nitric acid were added and samples were sonicated for 30 minutes. Two mL of hydrogen peroxide were carefully added and the mixture was sonicated for 15 minutes. This pre-digestion step was required to avoid foam formation and evolution of gasses resulting from the high content of carbohydrates in the samples, that could produce losses by overpressure inside the vessels during the digestion step. After that, the final volume was made to 10 mL using ultrapure water, to achieve the manufacture conditions to control the temperature, and vessels were introduced inside the microwave oven. The following program was run: 500 W exit power, 3 min to reach 85 °C, 9 min to reach 145 °C, 4 min to reach 180 °C, 15 min at 180 °C and 25 min at 30 °C. After cooling, the vessels were placed again in the ultrasound water bath in order to remove nitrous vapours and the resultant solutions were transferred to plastic flasks and brought to 25 mL with water. Two blank digestions were subjected to the same treatment for every set of samples.

2.4.2. Dry ashing procedure. Samples were accurately weighed (1.0 g) inside dry, clean glass 250 mL beakers, treated with 5 mL of HNO_3 50% (v/v), and evaporated to dryness in a sand bath. Then beakers were transferred inside a temperature-controlled electric muffle furnace and the following program was run: 5 hours to reach 450 °C, 12 hours at 450 °C (overnight) and 3 hours to reach room temperature. If carbon particles remained, the black residue was treated again with 5 mL HNO_3

50% (v/v) and it was dried and reashed. White ashes were moistened with 1 mL water and dissolved with 9 mL of HCl 10% (v/v). Resultant solutions were transferred to plastic flasks and brought to 20 mL of final volume. Four blank digestions were subjected to the same treatment for every set of samples.

Alternatively, the aforementioned procedure of dry ashing was applied to samples previously treated with 2.5 mL of an ashing aid suspension containing 20% (w/v) $Mg(NO_3)_2 \cdot 6H_2O$ plus 2% (w/v) MgO.

2.5. ICP-OES determination

Samples were analysed by ICP-OES using the experimental conditions summarized in Table 1. Elements were measured at two analytical emission lines for comparative purposes (Table 2). The calibration standards were prepared daily by diluting the stock ones in 1% (v/v) HNO_3. The calibration ranges were from 0.05 to 5 mg L^{-1} for Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Se, Sr, Ti, Tl, V and Zn, from 0.1 to 1.0 mg L^{-1} for Ce, La, Nd, Pr and Sn, from 0.02 to 0.2 mg L^{-1} for Dy, Er, Eu,

Table 2 Selection of emission lines for the analysis of supplementary foods by ICP-OES

	Wavelength 1					Wavelength 2					
	nm	Calibration line (I: cps, C: μg mL^{-1})	R^2	S/N^a	RSD^b	nm	Calibration line (I: cps, C: μg mL^{-1})	R^2	S/N^a	RSD^b	$C_1/C_2{}^c$
Al	396.153	$I = 124\,585C - 565$	0.9998	221	0.7	309.271	$I = 73\,835C - 2756$	0.998	27	0.5	0.6
As	188.979	$I = 2168C + 6$	0.9997	351	1.7	193.696	$I = 2176C + 19$	0.9997	115	3.9	0.9
B	249.772	$I = 93\,631C + 544$	0.9999	172	0.2	249.677	$I = 47\,748C + 430$	0.9998	111	0.2	1.0
Ba	493.408	$I = 3\,414\,685C + 2397$	0.9997	1425	4.2	233.527	$I = 141\,897C + 620$	0.9999	229	0.2	1.1
Be	313.107	$I = 2\,490\,370C + 8658$	0.9997	288	4.3	313.042	$I = 5\,443\,018C + 51\,432$	0.9996	106	4.4	1.0
Bi	223.061	$I = 6916C + 22$	0.9998	312	2.1	306.766^d	$I = 21\,763C - 388$	0.998	56	1.4	0.3
Ca	317.933	$I = 13\,209C - 9973$	0.9995	1	4.4	396.847	$I = 499\,808C + 719\,389$	0.998	1	2.7	1.0
Cd	214.440	$I = 101\,252C + 499$	0.9998	203	0.2	228.802	$I = 53\,439C + 166$	0.9999	322	0.2	0.9
Ce	413.764	$I = 85\,022C + 335$	0.9990	254	0.8						—
Co	238.892	$I = 107\,148C + 541$	0.9999	198	0.3	228.616	$I = 36\,242C + 204$	0.9997	178	1.1	1.0
Cr	267.716	$I = 90\,528C + 600$	0.9999	151	0.1	205.560	$I = 46\,667C + 233$	0.9999	200	0.5	1.0
Cu	327.393	$I = 199\,060C + 497$	1.0	400	0.2	324.752	$I = 311\,128C + 1889$	0.9999	165	0.1	1.0
Dy	353.170	$I = 390\,215C + 887$	0.9997	440	1.6						—
Er	337.271	$I = 243\,061C + 79$	0.9996	3088	1.1	349.910	$I = 181\,657C + 804$	1.0	226	1.3	1.0
Eu	412.970	$I = 1\,171\,013C + 1230$	0.9990	952	0.7	381.967	$I = 988\,063C + 1231$	0.9991	803	0.7	1.0
Fe	239.562	$I = 119\,673C + 790$	0.9998	152	0.4	238.204	$I = 90\,626C + 1112$	0.9998	82	0.5	1.0
Gd	342.247	$I = 135\,869C + 184$	0.9997	739	1.7	336.223	$I = 97\,168C - 114$	0.9999	856	3.0	0.9
Ho	345.600	$I = 261\,456C + 17$	1.0	15 269	0.7	339.898	$I = 116\,605C - 31$	0.9999	3780	1.5	1.0
K	766.490	$I = 1637C - 1939$	0.9990	1	4.3						—
La	408.672	$I = 437\,857C + 1938$	0.9990	226	0.9	379.478	$I = 263\,002C + 1488$	0.9993	177	1.0	0.9
Li	670.784	$I = 74\,982C + 103$	0.9997	726	4.2	610.362^d	$I = 9896C - 33$	0.9994	299	0.5	1.2
Lu	261.542	$I = 814\,616C + 86$	0.9999	9451	0.8	291.139	$I = 150\,627C - 18$	1.0	8338	1.7	1.0
Mg	285.213	$I = 39\,718C - 4158$	0.9998	10	4.1	279.077	$I = 1242C + 135$	0.9998	9	2.6	1.0
Mn	259.372	$I = 817\,578C + 4196$	0.9998	195	4.5	257.610	$I = 475\,879C + 2485$	0.9998	191	4.5	1.0
Mo	202.031	$I = 30\,675C + 131$	0.9997	235	2.5	203.845	$I = 14\,736C + 57$	0.9997	257	2.0	1.0
Na	588.995	$I = 22\,878C - 31\,052$	0.9996	0.7	4.4	589.592	$I = 12\,121C - 5146$	0.9996	2	2.6	0.9
Nd	401.225	$I = 96\,432C + 820$	0.9998	118	1.0	406.109	$I = 107\,355C + 530$	0.9990	203	0.7	0.9
Ni	231.604	$I = 36\,576C + 252$	0.9998	145	0.3	232.003	$I = 22\,332C + 89$	1.0	251	2.4	1.1
Pb	220.353	$I = 8072C + 30$	0.9997	271	3.0	217.000	$I = 1893C + 9$	0.9999	207	2.7	0.7
Pr	414.311	$I = 118\,692C + 696$	0.9990	171	0.9	390.844	$I = 92\,867C - 962$	0.9990	96	1.2	1.0
Sc	361.383	$I = 1\,206\,738C + 270$	0.9991	4466	0.7	357.253	$I = 1\,101\,433C + 1608$	0.998	685	0.7	0.9
Se	196.026	$I = 2366C + 20$	0.9995	121	2.0	203.985	$I = 2034C + 8$	0.9997	260	2.4	0.9
Si	251.611	$I = 36\,147C - 1626$	0.9990	22	1.4	212.412	$I = 9257C - 422$	0.9990	22	0.2	—
Sm	359.260	$I = 68\,104C - 28$	0.9997	2427	3.0	442.434^d	$I = 109\,387C - 395$	0.9990	277	0.7	0.4
Sn	189.927	$I = 34\,681C + 101$	0.9998	344	0.5	235.485^d	$I = 15\,889C + 44$	0.9999	362	0.6	0
Sr	407.771	$I = 1\,834\,886C + 22\,753$	0.9990	81	1.6	421.552	$I = 784\,536C + 10\,985$	0.9990	71	1.5	1.0
Tb	384.873	$I = 336\,608C + 590$	0.9995	571	0.8	350.917	$I = 113\,239C + 268$	0.9997	422	2.2	1.0
Ti	336.121	$I = 523\,555C + 859$	1.0	610	0.1	334.940	$I = 711\,548C + 1824$	0.9999	390	0.01	1.0
Tl	190.801	$I = 4311C + 53$	0.9997	82	1.6	276.787^d	$I = 3763C + 30$	0.9998	126	0.1	—
Tm	313.126	$I = 241\,665C + 323$	0.9999	748	1.5	346.220	$I = 175\,249C - 310$	0.9991	566	1.5	0.9
V	290.880	$I = 115\,307C + 52$	1.0	2218	0.5	310.230^d	$I = 330\,064C + 1191$	0.9993	277	0.5	0.8
Y	324.227	$I = 592\,543C + 103$	0.9999	5766	1.4	371.029	$I = 1\,108\,046C + 1346$	0.9993	823	1.0	1.0
Yb	328.937	$I = 2\,415\,308C + 2181$	0.9992	1107	0.9	369.419	$I = 771\,129C + 200$	0.9999	3848	1.0	1.0
Zn	213.857	$I = 104\,528C + 844$	0.9999	124	0.2	206.200	$I = 42\,016C + 342$	0.9999	123	0.1	1.0

[a] Signal to noise ratio S/N. [b] Relative standard deviation corresponding to three replicate analyses of one spiked supplementary food sample. [c] Ratio between concentrations obtained for a spiked supplementary food sample, employing both studied wavelengths. [d] Wavelengths indicated as d correspond to emission profile in sample interfered.

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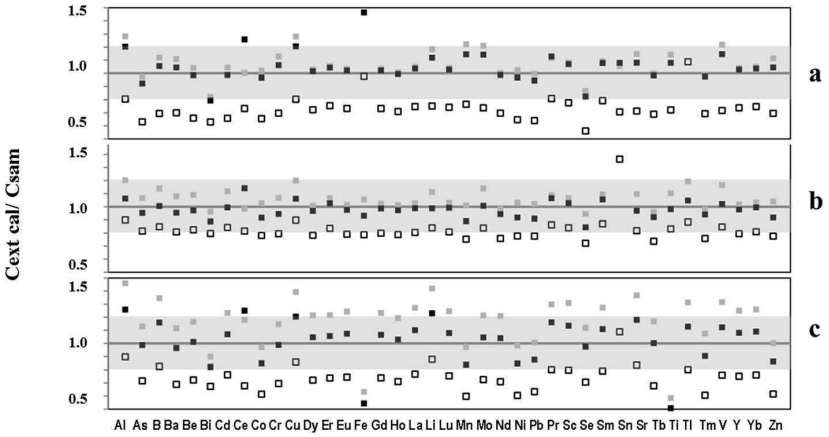

Fig. 1 Ratio between concentrations of elements obtained by ICP-OES using external calibration (Cext cal) and standard addition methods (Csam) using or not the internal standard of a supplementary food sample after (a) microwave acid digestion, (b) dry ashing or (c) dry ashing with ashing aid. ICP-OES measurements in all the cases without using internal standard (□), with Re (■) or Ru (■).

Gd, Ho, Lu, Sm, Sc, Tb, Tm, Yb, and Y and from 1 to 80 mg L^{-1} for Ca, K, Mg and Na. Rhenium 1.0 mg L^{-1} and Ru 1.0 mg L^{-1} were employed as ISs.

2.6. Quality control

Each sample was prepared and measured in triplicate. Blank digestions were subjected to the same treatment for every set of samples and measured together. Additionally, control standards were measured every 10 independent sample measurements. Reference material NIST 1573a tomato leaves were employed in order to evidence any bias.

3. Results and discussion

3.1. Analytical performance of ICP

Robust plasma conditions involve the capability of the plasma to accept changes in matrix samples, atomization and excitation conditions without changing its response. Robustness, typically indicated by ionic-atomic emission intensity ratio Mg II 280 nm/Mg I 285 nm, may be obtained when a high RF is used together with a low carrier gas flow rate and a moderate solving loading.[12]

Instrumental conditions were previously adjusted through the aspiration of Mg solution. Software devices automatically optimized Mg II 280 nm/Mg I 285 nm at a RF power 1.3 kW, carrier gas flow rate 0.2 L min^{-1} and sample aspiration rate 1.1 mL min^{-1}.

3.2. Selection of emission lines

When the sample composition is unknown, one of the first aims of the analysis step is to determine appropriate emission lines that provide an adequate sensitivity and a good selectivity without problems of spectral interference that usually arise from ICP-OES due to the multielement nature of the technique.

In this study two emission lines per analyte, which were presumed to be adequate according to manufacturer data and our own experience, were assessed in terms of sensitivity, selectivity and precision. A pull of microwave digested samples was divided into two portions. One portion was directly analysed and the other one was previously spiked with 0.5 and 0.1 μg mL^{-1} concentration levels for minor and trace elements, respectively. Spectra were carefully revised and the sloping background shift was corrected with manually selection of 2-points, one of each side of the profile of the analytical line. Table 2 shows the results of regression analysis of aqueous standards of the elements scrutinized in the pull sample, the relative standard deviation for the analysis in triplicate of the spiked sample and the ratio between concentrations obtained from emission spectra at both studied wavelengths. Therefore, attending to spectra shape, K 404.721, Li 610.362, Sm 442.434, Sn 235.485, Tl 276.787 and V 310.230 were clearly rejected. Al 309.271 and Bi 306.766 were also refused because small sensitivity leads to an inconsistent ratio between concentrations. The rest of lines were classified as selected wavelength or alternative wavelength looking for the highest sensitivity evaluated through the slope-values.

3.3. Study of matrix effects

Strategies to reduce matrix interference based on matrix separation, matrix matching and standard addition calibration are laborious and time consuming, therefore for the development of routine methods we have just employed an appropriate IS to compensate changes during transport and aerosol forming step, and the selection of the best dilution level of digested samples to reduce other matrix effects.

Elements with spectral lines with excitation and ionization energy similar to analyte ones are preferable to compensate plasma equilibrium and, because of that, spectral lines of Sc and Y, with intermediate energies, have been recognized as the universal IS.[2] However, to explore other alternatives, in this work, Re and Ru have been proposed for the analysis of digested food samples. Re 197.248 nm 1 μg mL^{-1} and Ru 240.272 nm 1

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μg mL^{-1} intensity monitored in aqueous standards get 20 000 cps and 21 000 cps, respectively, with a variation in the run sequence about 6%. The rhenium emission intensity in samples was suppressed by matrix effects in a nonsignificant way in the case of high diluted samples, around 10% in medium diluted samples, and 20% in undiluted digested samples. Analogously, ruthenium gave 15% and 25% signal suppression as a function of the final dilution of samples.

In order to check the capability of Re and Ru to correct matrix interference mineral concentrations of one sample were obtained with the use of external calibration (without IS) and internal calibration (Re and Ru) and compared with mineral concentrations obtained by using the standard addition method. Fig. 1 shows that the use of the IS is mandatory to avoid underestimated results when sample solutions were directly analyzed without additional dilution. It allows getting acceptable results in the case of using microwave acid digestion (a), excellent results with the dry ashing method (b), but it does not works with dry ashing with ashing aid (c). Regarding Ru or Re choice, both elements are adequate but Re provided better results than Ru whatever was the employed digestion method.

ESI 1,† analogous to Fig. 1 shows that the matrix effect in supplementary food analysis is reduced by increasing dilution. However dilution increases the limit of detection, hinders the

Table 3 Limit of detection and limit of quantification in supplementary food mineral analysis by ICP-OES after different sample treatments[a]

	LOD (μg g^{-1})		LOQ (μg g^{-1})	
	Microwave acid digestion	Dry ashing	Microwave acid digestion	Dry ashing
Al	3	1.2	11	4.2
As	1.0	0.07	3.4	0.22
B	0.4	0.14	1.3	0.47
Ba	0.04	0.010	0.12	0.032
Be	0.07	0.013	0.24	0.044
Bi	2	0.05	7	0.15
Ca	10	3	34	10
Cd	0.05	0.015	0.16	0.049
Ce	0.4	0.08	1.4	0.26
Co	0.2	0.03	0.8	0.08
Cr	0.6	0.9	1.9	3.1
Cu	1.1	0.09	3.7	0.30
Dy	0.12	0.02	0.40	0.07
Er	0.2	0.3	0.8	1.1
Eu	0.011	0.0010	0.036	0.0034
Fe	2	0.5	8	1.8
Gd	0.14	0.05	0.48	0.16
Ho	0.02	0.005	0.06	0.016
K	6	11	21	37
La	0.09	0.03	0.29	0.10
Li	0.14	0.07	0.48	0.23
Lu	0.007	0.0003	0.022	0.0009
Mg	1.0	2	3.2	7
Mn	0.05	0.05	0.17	0.17
Mo	0.3	0.05	1.1	0.16
Na	8	19	26	63
Nd	0.3	0.02	0.8	0.07
Ni	0.4	0.10	1.3	0.33
Pb	1.4	0.06	5.2	0.19
Pr	0.2	0.012	0.8	0.041
Sc	0.02	0.008	0.05	0.030
Se	1.0	0.9	3.5	3.0
Si	5	0.6	17	2.1
Sm	0.5	0.08	1.5	0.25
Sn	3	1.2	10	3.9
Sr	0.03	0.007	0.08	0.023
Tb	0.17	0.02	0.56	0.07
Ti	0.2	0.008	0.7	0.028
Tm	0.05	0.008	0.15	0.028
V	1.1	0.12	3.7	0.41
Y	0.03	0.003	0.09	0.010
Yb	0.02	0.002	0.05	0.007
Zn	1.7	1.2	5.8	3.9

[a] Note: LODs and LOQs were calculated as the concentration corresponding to signals equal to three-times and ten-times, respectively, the standard deviation of a blank solution related to a sample mass of 0.5 g.

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quantification of trace and ultratrace elements and may introduce mistakes in the calculation of final concentrations. From the results obtained from a pull of samples after wet digestion and dry ashing (ESI 2 and 3†), at three dilution levels: undilution, 1 : 1 dilution and 1 : 3 dilution, it can be concluded that data obtained for minor and major elements are comparable at the three dilution levels but the quantification of elements such as Co, Cr, Dy, Gd, La, Li, Mo, Se, Sr, V and Y strongly depend on the dilution factor. So dilution should be maintained at the lowest possible level. Therefore, the ICP analytical conditions selected for the analysis of food samples included the use of Re as the IS and direct measurement of undiluted digested samples in order to assure a good accuracy and sensitivity.

Table 4 Recovery of mineral elements on a spiked sample of supplementary foods analysed by ICP-OES after different sample treatments[a]

	Microwave acid digestion		Dry ashing	
Al	109^{50}	(22.8)	109^{25}	(16.0)
As	85^{5}, 99^{50}	(nq)	$114^{2.5}$, 102^{25}	(nq)
B	98^{5}, 109^{50}	(7.1)	$60^{2.5}$, 31^{25}	(6.2)
Ba	91^{5}, 108^{50}	(9.6)	$106^{2.5}$, 97^{25}	(10.2)
Be	103^{5}, 106^{50}	(nq)	$114^{2.5}$, 107^{25}	(nq)
Bi	nr^{5}, 98^{50}	(nq)	$65^{2.5}$, 99^{25}	(nq)
Ca	115^{500}	(620)	121^{225}	(700)
Cd	100^{5}, 103^{50}	(nq)	$111^{2.5}$, 105^{25}	(nq)
Ce	$104^{12.5}$	(nq)	$102^{6.25}$	(nq)
Co	96^{5}, 107^{50}	(nq)	$103^{2.5}$, 98^{25}	(nq)
Cr	82^{5}, 107^{50}	(5.1)	$90^{2.5}$, 102^{25}	(5.1)
Cu	101^{5}, 118^{50}	(11.1)	$108^{2.5}$, 106^{25}	(11.2)
Dy	$104^{2.5}$	(nq)	$96^{1.25}$	(nq)
Er	$129^{2.5}$	(nq)	$112^{1.25}$	(nq)
Eu	$107^{2.5}$	(nq)	$109^{1.25}$	(nq)
Fe	115^{50}	(121)	102^{25}	(125)
Gd	$79^{2.5}$	(nq)	$73^{1.25}$	(nq)
Ho	$106^{2.5}$	(nq)	$106^{1.25}$	(nq)
La	$103^{12.5}$	(nq)	$102^{6.25}$	(nq)
Li	117^{5}, 112^{50}	(nq)	$117^{2.5}$, 90^{25}	(1.6)
Lu	$106^{2.5}$	(nq)	$109^{1.25}$	(nq)
Mn	115^{50}	(110)	111^{25}	(117)
Mo	127^{5}, 111^{50}	(nq)	$107^{2.5}$, 98^{25}	(0.7)
Na	103^{500}	(60)		(1200)
Nd	$104^{12.5}$	(nq)	$121^{6.25}$	(nq)
Ni	110^{5}, 107^{50}	(nq)	$110^{2.5}$, 100^{25}	(1.3)
Pb	85^{5}, 102^{50}	(nq)	$108^{2.5}$, 99^{25}	(nq)
Pr	$109^{12.5}$	(nq)	$104^{6.25}$	(0.08)
Se	77^{5}, 89^{50}	(nq)	11^{25}	(nq)
Sm	$103^{2.5}$	(nq)	$102^{1.25}$	(nq)
Sn	60^{50}	(nq)	82^{25}	(nq)
Sr	107^{5}, 111^{50}	(5.1)	$112^{2.5}$, 102^{25}	(5.5)
Tb	$105^{2.5}$	(nq)	$95^{1.25}$	(nq)
Ti	100^{5}, 94^{50}	(1.0)	$102^{2.5}$, 78^{25}	(0.6)
Tm	$107^{2.5}$	(nq)	$107^{1.25}$	(nq)
V	95^{5}, 111^{50}	(nq)	96^{25}	(nq)
Y	$105^{2.5}$	(nq)	$108^{1.25}$	(nq)
Yb	$107^{2.5}$	(nq)	$110^{1.25}$	(nq)
Zn	101^{50}	(76)	80^{25}	(89)

[a] Note: data are the average of recovery results (%) corresponding to three independent analyses of one spiked supplementary food sample, performed using the primary recommended conditions for ICP-OES. The added concentration (μg g^{-1}) is indicated in superscript and the concentration sample (μg g^{-1}) in brackets. nq: not quantified.

3.4. Selection of pre-treatment procedure

Microwave acid digestion and dry ashing of dietetic and supplementary food samples were compared through the analytical parameters obtained for both methodologies. The use of $MgNO_3$/MgO as an ashing aid was rejected at the beginning of the study because of matrix effects which were not overcome and impurities in the reagents due to the use of high concentrations of Mg salts in blank solutions that probably found increased errors in final calculations (ESI 4†).

Limit of detection (LOD) and limit of quantification (LOQ) parameters were estimated as the analyte concentrations that give signals equivalent to three or ten times, respectively, the standard deviation of ten blank measurements and taking into account the amount of sample and the final dilution employed. LODs obtained after dry ashing methodology were smaller than those found by using microwave digestion with the exception of K, Mg and Na (Table 3). The obtained LODs from both employed methodologies were considered adequate to perform quantitative analysis of samples, except in the case of As and Se for which the sensitivity of ICP-OES is not excessively appropriate.

Analysis of spiked samples (Table 4) was used to validate the sample-preparation procedure followed by analytical measurements through ICP-OES. Recoveries for most elements were acceptable with values in the 85–115% range. However a significant bias was found for Se using the microwave procedure and for B, Bi and Se using the dry ashing due to the low sensitivity of ICP-OES for these elements. Additionally, analysis of the certified reference material NIST 1573a tomato leaves (Table 5) was done. Microwave acid digestion provided accurate data with recovery values between 73% and 108%. Unfortunately the dry ashing procedure provided overestimation of several elements, especially important in the case of B and Na, due probably to contamination from beakers.

The repeatability of the ICP-OES measurement, established from the RSD of three replicates of a digested sample, was under 4% for all elements (Table 2), thus it was considered suitable for correct analysis of foods. The repeatability of the proposed procedures was established from the RSD of five independent analyses of one sample. Good precision was obtained for most quantifiable elements, only Ti, which is present at a very low level, and Na, determined by using the dry ashing procedure, had an RSD above 10% (Table 6).

Finally, in Table 7, the results from the analysis of one supplementary food are shown. It is confirmed again that both methodologies provide acceptable results with exception of Na which is overestimated by the dry ashing procedure due to glassware contamination.

3.5. Evaluation of green parameters of the method

The greenness of the methodology employed was evaluated by using the analytical eco-scale proposed by Namiesnik.[13] From an initial scale of 100 points, the use of HNO_3, H_2O_2 and microwave energy for the treatment of samples involves 9

Table 5 Accuracy assessment of the proposed methodologies through the analysis of NIST tomato leaves 1573a[a]

		Microwave acid digestion		Dry ashing	
	Certified (μg g^{-1})	Found (μg g^{-1})	Accuracy (%)	Found (μg g^{-1})	Accuracy (%)
Al	598 ± 12	474 ± 30	79	750 ± 100	125
B	33.3 ± 0.7	35.4 ± 1.2	106	700 ± 300	2115
Ba	63	57.2 ± 1.7	91	60 ± 2	98
Ca	50 500 ± 900	43 100 ± 1800	85	45 000 ± 1400	89
Cd	1.52 ± 0.04	1.11 ± 0.05	73	0.72 ± 0.09	48
Ce	2	<2.0	—	3.16 ± 0.08	158
Co	0.57 ± 0.02	<1.0	—	<0.15	—
Cr	1.99 ± 0.06	<3	—	7.7 ± 0.3	387
Cu	4.70 ± 0.14	5.5–18.5	—	5.2 ± 0.4	110
Fe	368 ± 7	333 ± 7	91	464 ± 6	126
K	27 000 ± 500	25 300 ± 700	94	19 000 ± 1700	71
La	2.3	2.14 ± 0.06	93	2.1 ± 0.2	89
Mg	12 000	10 000 ± 300	83	10 200 ± 400	85
Mn	246 ± 8	241 ± 7	98	180 ± 40	72
Mo	0.46	<1.5	—	<0.25	—
Na	136 ± 4	40–131	—	4300 ± 900	3162
Ni	1.59 ± 0.07	<2	—	0.8 ± 0.3	50
Sr	85	91 ± 3	108	123 ± 4	145
Zn	30.9 ± 0.7	8.5–29	—	33 ± 4	107

[a] Found values expressed as the average of three independent analyses performed using the primary recommended conditions for ICP-OES.

penalty points whereas HNO_3, HCl and sand bath and muffle furnace energy involves 11 penalty points. The ICP-OES instrument, together with the use of Ar and standard solutions for calibration, involves 4 penalty points. Extra points from occupational hazard and produced wastes involve 3 penalty points. So the final score for the acid microwave assisted digestion method was 84 whereas for the dry ashing method it was 82 which means excellent green analysis in any case.

Finally, a muffle furnace has a capability for the simultaneous treatment of samples four times higher than the microwave oven; but to obtain white ashes it involves two or three runs. So it can be concluded that the time consumed in dry ashing treatment is higher than that used in microwave oven-assisted digestion.

Therefore analytical characteristics show that both proposed methodologies are suitable for the determination of most of the studied elements in supplementary food samples. Total destruction of organic matter and the use of diluted acids led to

Table 6 Precision for the methods proposed for supplementary food analysis by ICP-OES[a]

	Microwave acid digestion	Dry ashing	Concentration level (μg g^{-1})
Al	3	5	10–100
B	3	8	1–10
Ba	4	3	1–10
Ca	4	1	100–1000
Cu	5	4	1–10
Fe	9	11	10–100
K	4	13	>1000
Mg	5	4	>1000
Mn	4	2	10–100
Mo	6	2	<1
Na	2	41	10–100
Sr	4	3	1–10
Ti	17	33	<1
Zn	3	3	10–100

[a] Note: precision expressed as the relative standard deviation (%) of five independent analyses of a supplementary food sample, performed using the primary recommended conditions for ICP-OES.

Table 7 Effect of the digestion method of supplementary foods on concentrations (mg kg^{-1}) obtained by ICP-OES

	Microwave acid digestion[a]	Dry ashing[b]
Al	22.8 ± 1.7	16.0 ± 0.9
B	7.08 ± 0.07	6.2 ± 0.5
Ba	9.6 ± 0.4	10.2 ± 0.3
Ca	620 ± 19	694 ± 6
Cr	5.1 ± 0.5	5.1 ± 1.1
Cu	11.1 ± 0.3	11.4 ± 0.4
Fe	121 ± 6	126 ± 5
K	8000 ± 200	6400 ± 900
Li		1.6 ± 1.0
Mg	2300 ± 100	2500 ± 100
Mn	110 ± 4	117 ± 2
Mo		0.69 ± 0.02
Na	60 ± 2	1200 ± 500
Ni		1.3 ± 0.3
Sr	5.1 ± 0.2	5 ± 8
Ti	1.0 ± 0.3	0.6 ± 0.2
Zn	76 ± 2	88.9 ± 0.2

[a] 0.5 g of dry sample were digested in a microwave oven with HNO_3 and H_2O_2. The final volume was made to 25 mL. [b] 1 g of dry sample was dry ashed in a muffle furnace. Ashes were diluted in HCl and the final volume was made to 20 mL.

the lowest LODs by using the dry ashing method but the accuracy and repeatability on Na determination are the main drawbacks due to the material employed. However, for the microwave assisted digestion method, accurate results in a fast way can be obtained and for that reason it was our method of choice for the analysis of supplementary food samples.

4. Conclusions

The ICP-OES method developed and validated for the analysis of supplementary food samples is simple and reliable for routine analysis of major, trace and ultratrace elements. Although acceptable results were obtained by using microwave digestion and dry ashing pre-treatments, the first one is recommended because of its improved accuracy and time saving. Calibration using Re as an IS avoids matrix interference even when no additional dilution of digested samples was performed, therefore the sensitivity of the analysis was not reduced. The precision of the method with RSD values lower than 10% for most of the elements considered was adequate. The methodology was successfully applied to the determination of the mineral profile of supplementary foods showing that the dynamic range of the method covers the range of concentrations found in these products, and low limits of detection, which ensures the absence of toxic elements.

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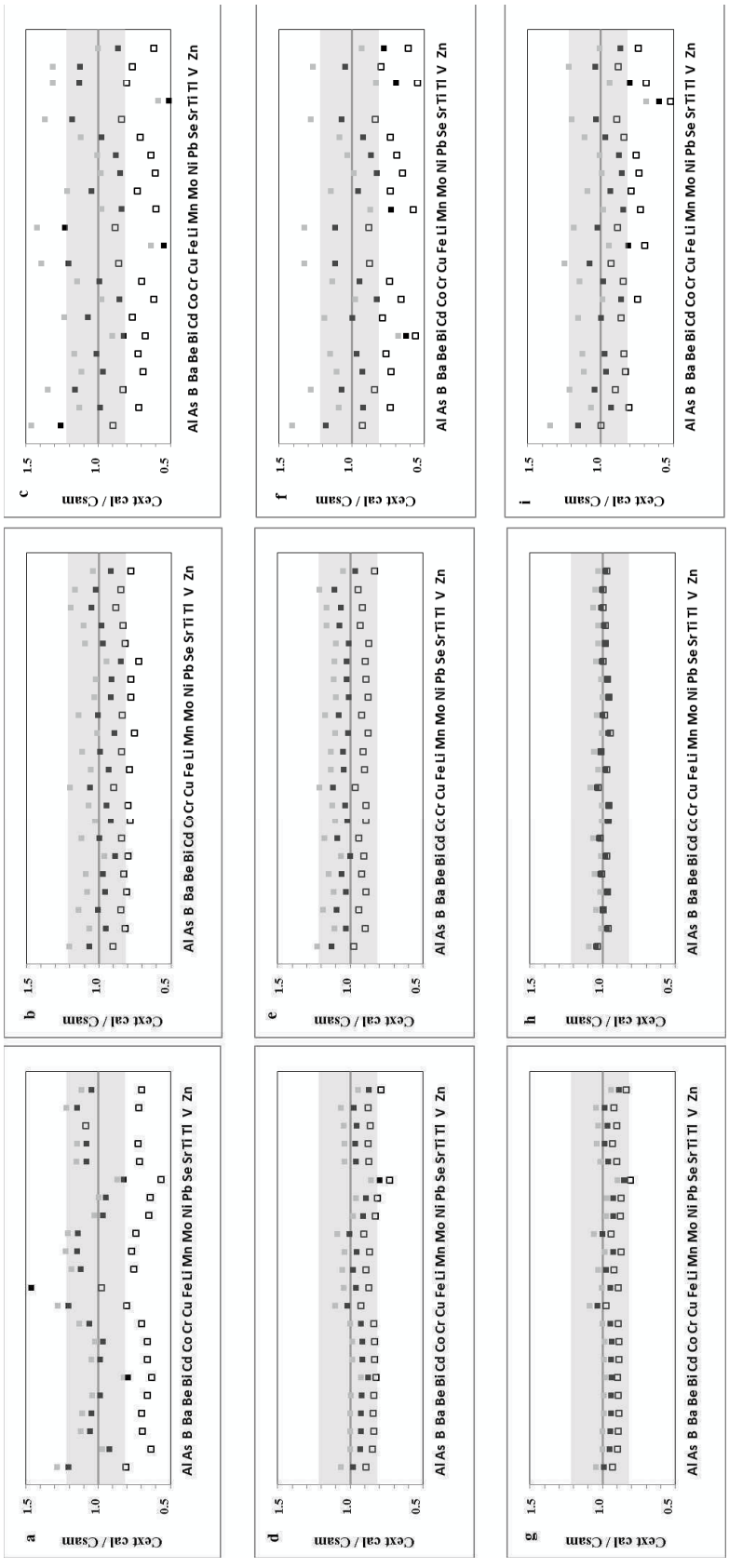

Supplementary 1. Ratio between concentrations obtained by using external calibration ($C_{ext\ cal}$) and the standard addition method (C_{sam}) of a supplementary food sample after a) microwave acid digestion-undiluted sample, b) dry ashing-undiluted sample, c) dry ashing with ashing aid - undiluted sample, d) microwave acid digestion-dilution 1:1, e) dry ashing- dilution 1:1, f) dry ashing with ashing aid –dilution 1:1, g) microwave acid digestion-dilution 1:3, h) dry ashing- dilution 1:3, i) dry ashing with ashing aid –dilution 1:3. ICP-OES measurements in all the cases without using internal standard (□), with Re (■) or Ru (■).

Supplementary 2. Effect of dilution factor of digested supplementary foods on concentrations (mg kg^{-1}) obtained by ICP-OES after microwave digestion

	undiluted	1:1	1:3
Al	21.6	21.2	21.2
B	5.5	5.5	5.3
Ba	7.0	6.8	6.3
Ca	692	688	682
Co	0.6	0.4	nd
Cr	4.4	4.3	3.8
Cu	8.2	8.0	7.8
Dy	nd	nd	nd
Er	1.3	1.2	1.1
Fe	95.3	96.7	98.4
Gd	0.2	nd	nd
K	8010	7860	7750
La	1.2	0.9	0.1
Li	nd	nd	nd
Mg	2250	2220	2210
Mn	83.5	83.9	85.1
Mo	0.1	nd	nd
Na	80	80	80
Pb	6.1	6.5	6.3
Se	nd	nd	nd
Sn	37.9	40.1	39.3
Sr	3.5	2.8	nd
Ti	0.7	0.6	0.4
V	1.0	1.0	nd
Y	nd	nd	nd
Zn	57.2	59.4	61.1

0.5 g of dry sample was digested in microwave oven with HNO_3 and H_2O_2. Final volume was made to 25 mL. 50 mL or 100 mL.

Supplementary 3. Effect of dilution factor of digested supplementary foods on concentrations (mg kg^{-1}) obtained by ICP-OES after dry ashing

	undiluted	1:1	1:3
Al	3.1	3.0	nd
B	nd	nd	nd
Ba	7.0	7.0	6.8
Ca	602	629	654
Co	0.5	0.5	nd
Cr	0.6	0.6	nd
Cu	7.6	7.4	7.2
Dy	0.1	0.1	nd
Er	0.6	0.6	0.5
Fe	68.0	68.7	69.2
Gd	0.2	0.2	0.3
K	5000	5040	5000
La	0.9	0.9	0.7
Li	1.0	0.9	0.8
Mg	-	-	-
Mn	79.0	80.0	80.5
Mo	0.3	nd	nd
Na	1260	1300	1320
Pb	5.9	6.0	6.1
Se	3.1	3.5	4.9
Sn	27.4	31.1	28.1
Sr	3.4	3.2	2.7
Ti	0.2	0.1	0.1
V	0.6	0.7	0.5
Y	0.0	0.1	nd
Zn	58.7	60.1	61.9

1 g of dry sample was dry assed in muffle furnace. Ashes were diluted in HCl and final volume was made to 20 mL. 40 mL or 80 mL.

Supplementary 4. Concentration of minerals in method blanks obtained from different sample treatments in supplementary foods analysis by ICP-OES

	Microwave acid digestion	Dry ashing	Dry ashing with ashing aid
Al	nq	nq	17.3 ± 0.3
B	nq	nq	30.3 ± 0.6
Ca	nq	nq	490 ± 4
Fe	nq	nq	7.9 ± 0.7
Mg	nq	nq	46700 ± 200
Mn	nq	nq	1.100 ± 0.004
Na	64.3 ± 0.7	nq	Nq
Ni	nq	nq	17.9 ± 0.3
Sr	nq	0.020 ± 0.002	1.503 ± 0.018
Ti	nq	0.068 ± 0.003	0.309 ±0.017

Data expressed in μg g^{-1} as the mean (± standard deviation) of three independent analysis performed using the primary recommended conditions for ICP-OES.

2. Determinación de mercurio

2.1. Evaluación de métodos para la determinación de mercurio en setas

Evaluation of greenness of methods for the determination of mercury in mushrooms

Manuela Ruiz de Cenzano, M.Luisa Cervera,
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Evaluation of Greenness of Methods for the Determination of Mercury in Mushrooms

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Abstract: In the present study, two analytical methodologies, one based on direct thermal degradation of samples followed by atomic absorption analysis (TDA-AAS), and another one, which consists on acid microwave-assisted digestion (MAD) followed by cold vapour atomic fluorescence determination (CV-AFS), have been applied to determine mercury in mushrooms. Both methodologies found the same concentration values, from 258 to 570 ng g^{-1} (dry weight), for nine samples. Analysis based on thermal degradation provided a limit of detection of 3 ng g^{-1} similar to that found by CV-AFS, and a relative standard deviation of 3 %, three times higher than that of CV-AFS. However, the direct analysis of untreated samples agrees well with green analytical principles because it involves less reagents consume and reduces waste generation. In fact, at analytical Eco-Scale, TDA-AAS procedure involves a green score of 92 points in front of the 59 points obtained for CV-AFS method.

Keywords: Analytical eco-scale, cold vapour, direct analysis, green analytical chemistry, mercury, microwave-assisted digestion, mushroom, thermal degradation.

1. INTRODUCTION

Mercury is considered one of the most hazardous pollutants due to its toxicity and accumulative character [1]. Food is the major route of exposure to mercury for humans and it is well known that seafood products represent the highest contribution on mercury intake. However there is a growing demand for analytical information about the presence of mercury in other foodstuffs [2].

Mushrooms consumption has become very popular in recent years due to their excellent flavor and nutritional properties, but they are able to accumulate relatively high concentrations of heavy metals. So many investigations have dealt with mercury content of mushrooms [3-5].

Modern methods for mercury trace determination are based on wet digestion with oxidizing acids or alkalis in combination with other reagents and additional energy treatment, followed by detection with cold vapour atomic absorption spectroscopy (CV-AAS), could vapour atomic fluorescence spectroscopy (CV-AFS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectroscopy (ICP-MS). Furthermore a direct technique of analysis based on thermal decomposition, mercury amalgamation and atomic absorption spectrometry (TDA-AAS) has been popularized as a fast alternative to do these determinations in last years. As can be seen in (Table **1**) some comparative studies focused on TDA-AAS reported advantages on this technique compared with other procedures [3, 6-12]. The main aspects of these comparative studies are based on the analytical figures of merit of the methodologies assayed together with the practical aspects, like speed or cost of analysis but there is a lack of a deep evaluation of green parameters of used methods.

Nowadays there is an increasing consciousness on green analytical chemistry (GAC) [13-15] that makes mandatory the quantitative evaluation of green features of methods apart from typical analytical ones and thus recently, Prof. Namiesnik has proposed the 12 principles of GAC [16] from green chemistry principles postulated by Anastas and Warner [17], and has suggested an Eco-scale as metric approach to quantitatively evaluate method greenness [18].

The purpose of our study has been the development, application and evaluation, from a green analytical point of view, of two modern alternatives for mercury trace determination in mushrooms, based on acid digestion followed by CV-AFS and direct analysis by TDA-AAS, respectively.

2. MATERIAL AND METHODS

2.1. Instruments and Apparatus

Mushroom samples were freeze-dried with a Telstar Cryodos system (Barcelona, Spain).

In sample treatment previous to CV-AFS determination, an ultrasound water bath from Selecta (Barcelona, Spain), of 350 mL volume operated at 360 W power and 50 Hz frequency was employed, and digestions were performed using a microwave labstation Ethos SEL from Milestone (Sorisole, Italy) operated at 1000 W. The microwave vessels were cleaned with sub-boiling HNO_3 in the Trace-Clean apparatus from Milestone. Possible traces of mercury were removed from solutions by using nitrogen stream, obtained from a generator Claind (Lenno, Italy).

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Table 1. Comparative studies between modern methods for Hg trace determination published in the literature.

Reference	Sample/technique	Results	Conclusions
[3]	Mushrooms TDA-AAS and ICP-OES		ICP-OES gives inaccurate and imprecise results
[6]	Dietary supplements TDA-AAS and ICP-MS	0.002 - 56 μg g^{-1} (10 samples)	Similar results. TDA-AAS was much faster and safer and required no microwave digestion
[7]	Fish tissues TDA-AAS and CV-AAS	0.9 - 1500 ng g^{-1} (72 samples)	Equivalent results. TDA-AAS gives better Hg recoveries, is faster and produces no waste, requires less sample and has low risk of contamination
[8]	Raisins, nails, hair, coal, soil, carrot, tea, tobacco, tomato leaves, blood, tree rings TDA-AAS and TDA -AFS	~2 -~1000 ng g^{-1}	Similar results. TDA-AFS provides lower detection limits but is not currently commercially available
[9]	Fish muscle TDA-AAS, CV-AAS and CV-AFS	0.011 - 0.051 μg g^{-1} (7 samples)	Comparable results. TDA-AAS reduces potential dilution and calculation errors
[10]	Contaminated soil and plants TDA-AAS, ICP-OES and CV-AAS	5-250 mg kg^{-1}	Comparable values when samples masses are optimized for TDA-AAS. Favourable attributes are portability, no acid digestion and short time required for analysis but condensed water and dust accumulation in chambers should be controlled
[11]	Soil TDA-AAS and CV-AAS	3.34 mg kg^{-1}	Similar results. TDA-AAS did not require a correction factor, had a lower detection limit than CV-AAS, low uncertainty, uses few reagents and reduced waste
[12]	Soil and vegetable TDA-AAS and CV-AFS	653-2004 μg kg^{-1} (5 soil samples) 4.6-53.4 μg kg^{-1} (5 vegetable samples)	Comparable results. TDA-AAS provides a lower detection limit and a higher reproducibility than CV-AFS

CV-AFS system PSA 10.025 Millennium Merlin instrument (Orpington, Kent, UK), working at 29 VA was equipped with a high intensity source BDHCL (boosted discharge hollow cathode lamp) superlamp from Photron (Victoria, Australia).

Direct mercury determination, based on thermal degradation atomic absorption spectroscopy, was made using a Milestone apparatus DMA-80 operated at 1200W.

Ultrapure water, with a resistivity of 18.2 MΩ cm, was obtained from a Milli-Q system Millipore (Bedford, USA).

2.2. Reagents and Standards

1000 mg L^{-1} $HgCl_2$ certified standard stock solution was obtained from Merck.

Nitric acid 69% (w/v) and 35% (w/v) H_2O_2, both from Scharlau (Barcelona, Spain) were employed for sample digestion in the presence of 2000 mg L^{-1} $K_2Cr_2O_7$ solution obtained from Panreac (Barcelona, Spain). For generating the vapour of mercury, prior to the AFS measurement, the reductant agent used was 2% (w/v) $SnCl_2 \cdot 2H_2O$ in HCl. This solution was prepared by dissolving the salt from Merck (Darmstadt,Germany) in 10% (v/v) HCl Merck and then it was purged with a stream of nitrogen for 30 min.

All chemicals used were of the highest purity available and all solutions were prepared in ultrapure water. Argon C-45 and oxygen C-45 were supplied by Carburos Metálicos (Barcelona, Spain).

2.3. Experimental Procedures

2.3.1. Sample Collection, Transport and Storage

Samples of mushrooms (*Lactarius deliciosus*), originated from spatially distant background areas across Spain, were purchased at a local market in Valencia (Spain) and transported to the laboratory. Fresh samples were carefully washed, the edible parts were cut and crushed and they were freeze-dried for approximately 48 h at a chamber pressure of 0.05 mbar. Dried samples were crumbled and pulverized with a domestic mill until to have an homogeneous mixture. Powdered samples were stored in polyethylene bottles and preserved in desiccator till analysed.

Certified reference material TORT-2 (lobster hepatopancreas) was provided by National Research Council of Canada.

2.3.2. Microwave-assisted Digestion and CV-AFS Determination

Representative dried samples of 0.5 g were weighed inside teflon vessels. Pre-digestion step was carried out in an ultrasound water bath for 30 minutes with 4 mL of HNO_3 and 2 mL of H_2O_2. Then, 4 mL of H_2O were added. Microwave assisted digestion program included five steps: 1) 3

min to reach 85 ºC, 2) 12 min to reach 145 ºC, 3) 10 min to reach 180 ºC, 4) 15 min at 180 º C, 5) cooling down to 30 º C for 25 min. Then samples were diluted to 25 mL with ultrapure water. A blank solution was subjected to the same treatment for every set of samples.

An aliquot of 4 mL, from the resultant digested solution, was transferred to a 50 mL volumetric flask, adding 100 µL of 2000 mg L^{-1} $K_2Cr_2O_7$ solution and 2.5 mL of HNO_3. Each sample was analyzed in triplicate by CV-AFS using the experimental conditions established before [19] (see Table **2**). Hg concentration was determined using external calibration with Hg (II) standard solutions prepared daily by sequential dilution. Freshly calibration standards from 0.2 to 1.6 µg L^{-1} in HNO_3 5 % (v/v) and $K_2Cr_2O_7$ 4 mg L^{-1} were used. Blank solutions were prepared by adding the amount of HNO_3 and $K_2Cr_2O_7$ employed for preparation of standard solutions.

Table 2. Operating conditions for the CV-AFS and TDA-AAS determination of Hg in mushrooms.

CV-AFS		**TDA-AAS**		
		Step	**Time (s)**	**Temperature (ºC)**
Gain	100	Dry	60	300
Delay time (s)	15	Decomposition	180	850
Analysis time (s)	40	Amalgamation	12	
Memory time (s)	60	Purge	60	
Carrier flow rate (mL min^{-1})	9	Recording	30	
Sample flow rate (mL min^{-1})	9			
$SnCl_2$ flow rate (mL min^{-1})	4.5	Oxygen flow rate (mL min^{-1})		200
Carrier gas flow rate (mL min^{-1})	250			
Dryer gas flow rate (L min^{-1})	2.5			
HNO_3 (mol L^{-1})	0.8			
$SnCl_2$ % (w/v)	2			

2.3.3. Direct Analysis by Thermal Decomposition, Amalgamation and Atomic Absorption Spectroscopy

A representative dried sample of 0.05 g was directly weighed inside a nickel boat and introduced into the DMA-80 system. The sample was initially dried and then thermally decomposed in a continuous flow of oxygen. Combustion products were trapped in a catalyst hot bed. Sulphur oxides, nitrogen oxides and halogens, which can interfere the Hg analysis, were flushed out of the system and retained on an activated carbon tube. Hg (0) was selectively trapped on a gold amalgamator, subsequently desorbed for absorbance measurement and the measured vapour finally retained on the activated carbon tube. Operating parameters are summarized in Table **2**. Instrument calibration was verified every work session by the analysis of the certified reference sample.

3. RESULTS AND DISCUSSION

3.1. Development of the Microwave-assisted Digestion Procedure

The volatile nature of mercury and its low concentration level in mushroom samples requires a high care in selecting the appropriate material and its cleaning procedure. Otherwise, any losses or contamination might cause serious systematic errors in the determination. Thus teflon microwave vessels were cleaned with sub-boiling HNO_3 in the Trace-Clean instrument and laboratory glassware was treated with 10 % (v/v) HNO_3 (15-30 min) and 10 % (v/v) HCl (overnight) to avoid cross contamination [19].

Pre-digestion step at room conditions was carried out to avoid a high evolution of gases and leaks during digestion under pressure inside the microwave oven. Experimental observation showed the importance of adding H_2O_2 carefully. Microwave assisted digestion program was set according with our previous experience in digestion of vegetable and rice matrices [19]. The vessels were opened and vented overnight before dilution to of minimize the amount of nitrous vapours.

3.2. Development of CV-AFS Methodology

Microwave digestion with an acid and oxidant medium provides clear extracts where mercury is supposed to be free and stable as Hg (II). However, a sample treatment is necessary to avoid possible interferences caused by dissolved organic matter or nitrous oxides. Different approaches can be used like increasing the acidity of solutions or using strong oxidizing agents or diluted samples.

In this work, to overcome matrix interferences during CV-AFS, oxidant mixtures, like $KBr/KBrO_3$, $KMnO_4$ or $K_2Cr_2O_7$ were used, being employed 5 % (v/v) HNO_3, 5% or 25% (v/v) HCl as reaction medium. On the other hand, different dilution factors were assayed by taking different volumes of digested solution of 4 mL, 5 mL or 7 mL for a final volume of 50 mL.

In the present study, the use of HCl was rejected because it clearly decreased the analytical sensitivity, especially when 25% (v/v) was employed. Regarding sampling dilution, 4 mL of digested mushroom were diluted in HNO_3 with $K_2Cr_2O_7$, to a final volume of 50 mL. The residual matrix effect was corrected by dividing the sample signals by a proportional coefficient of 0.86 in order to provide a good comparability with the external calibration.

3.3. Experimental for TDA-AAS Methodology

Preliminary studies were focussed on the amount of sample ranging from 10 till 150 mg (Fig. **1**). It was concluded that there is no influence of the amount of sample on the obtained concentration of mercury in this range. So 50 mg of sample were selected as an adequate amount in terms of both, reproducibility and representativity.

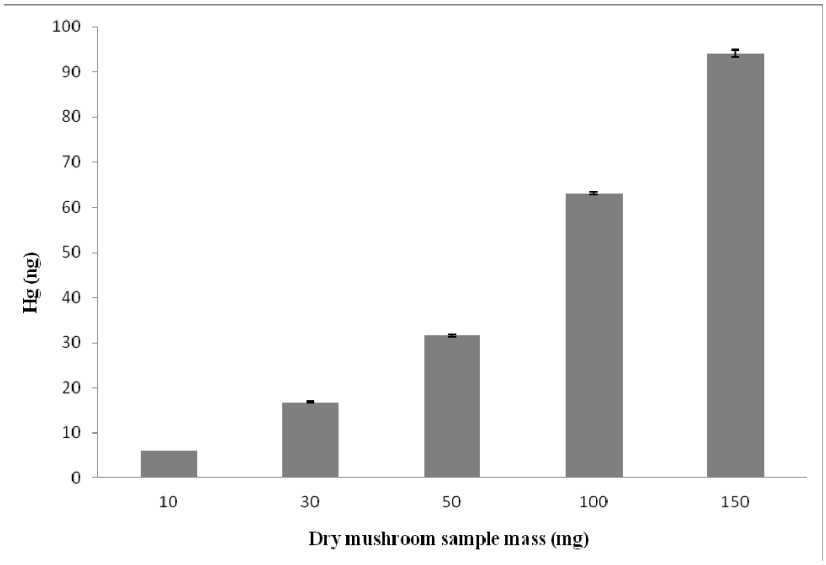

Fig. (1). Influence of sample mass on Hg measurement by TDA-AAS.

3.4. Analysis of Mushrooms Samples

Table **3** summarizes Hg concentration data obtained from nine mushroom samples. As it can be seen CV-AFS method provided a Hg range from 258 to 645 ng g^{-1} and TDA-AAS from 280 to 649 ng g^{-1}. Additionally, there is a clear correlation between data found by both methodologies, CV-AFS (y) and TDA-AAS (x), being the correlation equation y = (1.03 ± 0.04) x – (16 ± 17) with r = 0.990. Experimental Student's t-values of 0.76 and 0.90 were found for the comparison of the slope with 1 and the intercept with 0 respectively, for a probability level of 95% and 3 freedom degrees, being in both cases lower than the theoretical value of 2.45, which indicates that both assayed methods provide comparative results without any systematic error nor a blank effect.

Table 3. Mercury concentration in mushrooms.

Sample	CV-AFS	TDA-AAS
1	360 ± 20	357 ± 7
2	463 ± 12	441 ± 9
3	480 ± 20	487 ± 13
4	640 ± 60	649 ± 18
5	258 ± 14	280 ± 20
6	332 ± 19	340 ± 30
7	560 ± 40	570 ± 20
8	300 ± 30	320 ± 30
9	425 ± 3	416 ± 5

Data expressed in ng g^{-1} (dry weight) (average ± 3 replicate analysis).

3.5. Comparison of Analytical Figures of Merit

The limit of detection LOD established using the 3s criterion, the limit of quantification LOQ determined with 10s criterion and the precision of the methods evaluated as the relative standard deviation (RSD) of 6 replicates of a sample, are indicated in Table **4**. Comparatively the studied methodologies show very close analytical figures of merit and allows Hg determination at trace levels with a good precision. The obtained results for recovery studies with spiked samples at 400 ng g^{-1} (see Table **4**) and the analysis of a certified reference sample TORT-2 evidenced the comparable and good accuracy of both methods. In the analysis of the certified material CV-AFS provided a value of 0.284 ± 0.003 mg kg^{-1} Hg and TDA-AAS 0.280 ± 0.057 mg kg^{-1} for a certified value of 0.270 ± 0.060 mg kg^{-1} Hg.

3.6. Evaluation of Green Figures of Merit

From a green analytical point of view, the main aspects to be considered for a method evaluation involve all the analytical steps [13-15]. In the special case of spectroscopy methods [20, 21] it must be considered: i) the dangerous aspects of used reagents; ii) the energy consume related to the instruments and apparatus used; iii) the occupational hazard and iv) the amount of wastes provided by the method application. Prof. Namiesnik proposed a 100 points Eco-scale [18] to evaluate a method following the suggestion of Van-Aken in the organic field. From an initial score of 100, penalty points were introduced as a function of the deleterious effects of the method from the use of toxic or dangerous reagents, energy consume, hazards for operators and waste generated.

In the case of reagents consume, penalty points were established on the basis of pictograms employed in the Globally Hazardous System Classification and Labeling of Chemical (GHS) and signal words in a way that no pictograms involves no penalty, less severe hazard of each em-

Table 4. Analytical characteristics for CV-AFS and TDA-AAS determination of Hg in mushrooms.

	CV-AFS	**TDA-AAS**
Calibration range	0.2-1.6 μg L^{-1}	0.5-20 ng (low level); 20-1000 ng (high level)
Calibration equation	$y = (475 \pm 3)\, x + (0.7 \pm 3.0)$	$y = (6.31 \pm 0.01)\, 10^{-2}\, x - (1.10 \pm 0.01)\, 10^{-3}\, x^2$ $y = (9.0 \pm 0.1)\, 10^{-4}\, x - (3.0 \pm 0.1)\, 10^{-7}\, x^2$
Correlation coefficient	0.9998	1.0; 1.0
LOD (ng g^{-1})	2.0	3.3
LOQ (ng g^{-1})	6.4	10.0
RSD (%), n = 6	1.2	3.0
Recovery (%), 400 ng g^{-1} HgCl added	102 ± 6	97 ± 4

ployed reagent means 1 point penalty and severe hazard of each used reagent involves 2 points penalty. So in the case of CV-AFS evaluation, the sample preparation is the key step [20] being introduced 4 penalty points because of the use of HNO_3 and H_2O_2, 10 extra penalty points for using $K_2Cr_2O_7$ plus additional 4 points for HCl and 1 for $SnCl_2 \cdot 2H_2O$ employed for vapour Hg generation, and 1 point for the consume of Ar as transport gas (see Table **5**). Additionally 4 penalty points must be added due to the use of the standard solutions.

On the other hand, the absence of sample treatment in TDA-AAS involves a total of 4 points penalty due to the use of O_2 as combustion gas, being unnecessary the frequent use of Hg solutions for calibration.

Regarding the energy consume of apparatus and instruments, modern microwave assisted digestion provides a significant reduction in the amount of sample required (GAC principle number 2) [16] and acid used. However the number of samples suitable to be treated simultaneously inside the microwave oven (10 samples) is reduced. So energy consumption is important involving 1 point penalty. Ultrasounds can process simultaneously many samples in a single run and because of that the energy input used per sample is very reduced involving no penalty. On the other hand, TDA-AAS involves the use of a 1200 W furnace, which is employed for 6 minutes time per sample, thus achieving a 120 W energy consume with a penalty of 1 point.

Taking into account the occupational hazard, sample pretreatment in CV-AFS method uses strong acids and oxidant reagents that could be dangerous for operators, thus involving 1 penalty point, while TDA-AAS is an hermetic process without penalization because a glass tube filled with an activated carbon absorbs mercury and other gas emissions generated during the sample combustion.

Considering the volume of waste to be treated (GAC principle 7), acid solution with traces levels of toxic elements are produced in CV-AFS. However, this can be drastically reduced to few grams of a solid waste after a treatment with Fe (III) and alkalinization with NaOH [23]. Additionally the presence of $Fe(OH)_3$ as the main component of the waste passivates toxic elements [24], being clear that Hg vapour generated in CV-AFS instrument is trapped in the activated carbon tube unit as it happens in TDA-AAS.

In short, the greenest way of analysis is the use of a direct analytical technique without sample treatment nor analyte derivatization (GAC principle 1). So it can be concluded that for trace Hg determination, by using TDA-AAS, minimal sample size is recquired (GAC principle 2), toxic reagents are avoided (GAC principle 11), a minimum amount of waste from Hg vapour generation is trapped in activated carbon tube (GAC principle 7), automated analysis is performed (GAC principle 5), and safety of the operator is clearly increased (GAC principle 12). So this methodology is free from environmentally deleterious side effects with a total score of 92 in the Eco-scale, which corresponds to an excellent green methodology in front of the 59 points provided by CV-AFS, which represents an acceptable green analysis.

4. CONCLUSIONS

Two alternative procedures for mercury determination in mushroom samples have been validated and applied to the analysis of natural samples. Typical analytical characteristics, like accuracy, sensitivity or precision are similar and obtained mercury concentrations were statistically comparable. From a green evaluation, it can be concluded that use of modern methods, like microwave-assisted digestion and sonication followed by CV-AFS, contribute to relatively minimize consumption of toxic reagents as compared with traditional methods. The passivation of wastes after measurement, using Fe(III) in alkaline medium provided acceptable green analysis results. However direct analysis by TDA-AAS is the best approach because it satisfies a great number of GAC principles: no sample treatment, minimal sample size, automated analysis, minimum waste, elimination of toxic reagent and safety of the operator and this must be the method of choice in order to avoid environmental side effects.

Table 5. Evaluation of the greenness of CV-AFS and TDA-AAS mercury determination in mushrooms.

	CV-AFS	Penalty Points	TDA-AAS	Penalty Points
Reagents	HNO_3	4	Oxygen	4
	H_2O_2	4		
	$K_2Cr_2O_7$	10		
	HCl	4		
	$SnCl_2 2H_2O$	1		
	Argon	1		
	Hg standard solutions	4		
Instruments and apparatus	Freeze-drying instrument	1	Freeze-drying instrument	1
	Analytical balance	0	Analytical balance	0
	Ultrasounds	0	Furnace	1
	Trace clean	0	Spectrometer	0
	Microwave oven	1		
	Spectrometer	0		
Occupational hazard		1		0
Waste		10		2
Total penalty points		∑41		∑8
Analytical Eco-scale total score		59(acceptable)		92(excellent)

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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2.2. Determinación de mercurio en legumbres

Mercury content in legumes from Spain

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Mercury content in legumes from Spain

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Abstract

Total mercury has been quantitatively determined in a set of samples of most consumed legumes in Spain including beans, chickpeas, lentils and peas. The analytical methodology employed was based on direct thermal degradation of samples followed by atomic absorption spectroscopy analysis. Preliminary studies were focused on selecting the sample mass and instrumental dry time required. The limit of detection of the method was 0.021 ng g^{-1} and the relative standard deviation corresponding to three measurements varied between 3.8 and 19 %. Adequate accuracy was assured by the analysis of certified reference materials Fly Ash NIST 1633b and Rice Flour NIST 1589a with recoveries of 95.5 % and 118.4 %, respectively. Mercury levels in 20 legume samples were found in the range from 0.127 to 0.53 ng g^{-1}. The estimated weekly intake of mercury in Spain, via legume consumption ranged from 0.12 to 0.52 ng kg^{-1} body weight.

Keywords: Legumes; pulses; mercury; food safety; thermal degradation; mineral profile

INTRODUCTION

Mercury (Hg) is a local, regional and global pollutant that affects ecosystem and human health. It is considered one of the most hazardous pollutants due to its toxicity and accumulative character (Driscoll et al., 2013). Mercury interferes biological function of enzymes and proteins, due to the element affinity for sulfur, sulfhydryl and nitrogen groups, thus causing devastating effects, particularly in neuronal, vascular and adipose systems (Ynalvez et al., 2016). For humans, food is a major route of exposure to mercury. The Joint FAO/WHO Expert Committee on Food Additives has established a provisional tolerable weekly intake (PTWI) for total mercury of 4 μg kg^{-1} body weight (JECFA, 2011). The main intake of mercury occurs via fish consumption (Driscoll et al., 2013). However there is a growing demand on the knowledge of mercury concentration in other foodstuffs such as legumes, which have relatively low levels of mercury but are staple foodstuffs of all the regional diet around the world and can also make an important contribution in total mercury intake.

Legumes have a key socioeconomic role in countries such as India, Canada, Myanmar, China and Brazil. According to Food and Agriculture Organization of the United Nations, total production of pulses reached 73 million tons in 2011–13 and around 7 kg of pulses are consumed worldwide per person per year (FAOSTAT, 2016). Nutritionally, they are a good source of protein, carbohydrates, dietary fibers and minerals such as Cu, Fe, Mn, Mo, Ni and Zn (Gupta and Gupta, 2014; Gutiérrez-Uribe et al., 2016) and contribute to positive health impact in the prevention and management of diabetes (Singhal et al., 2014), cardiovascular disease (Marventano et al., 2016) and some types of cancer (Wang et al., 2013). However, damaging levels of harmful elements, such as Hg, can be present in legumes when they grow in contaminated soils or polluted areas (Balaji et al., 2000).

In the last years a number of published studies have been focused in the mineral content of legumes. Most of them use multielemental analysis technique such as neutron activation analysis (Miyamoto et al., 2000), X-rays (Vives et al., 2006), inductively coupled plasma optical emission spectroscopy (Santos et al., 2008) and mass spectroscopy (Koplik et al., 2002) in order to establish the mineral profile of legumes. Several works are focused on determining nutritional minerals Mg, K, Fe, Mn, Cu or Zn (Anzano and Ruiz-Gil, 2005; Sanchez et al., 2006; Zhou et al., 2006) which are present generally at part per million concentration; or selenium at parts per billion concentration (Sirichakwal et al., 2005). However only few papers deal with hazardous elements such as As, Cd, and Pb (Cabrera et

al., 2003; Matos-Reyes et al., 2010; Yebra and Cancela, 2005) and Hg (Waheed et al., 2003). To our knowledge data about mercury in legumes are very scarce. Samples of beans, chickpeas and lentils purchased from the local markets in Kashmir region were analyzed by Waheed et al. (2003) who reported a total Hg content of 32 ± 4 ng g^{-1}, 29 ± 3 ng g^{-1} and 33 ± 3 ng g^{-1}, respectively (Waheed et al., 2003).

The aim of the present work has been the determination of total mercury in most consumed legume varieties produced in Spain: beans, chickpeas, beans lentils and peas, using thermal–degradation-amalgamation atomic absorption spectroscopy (TDA-AAS). Results were assessed taking into account data about Spanish legume consume and Tolerable Weekly Intake established for mercury and compared with the occurrence of mercury in other foodstuffs. Obtained data were added to the general mineral profile known for legumes according to databases.

MATERIAL AND METHODS

A total of 20 legume samples covering 7 leguminose species were acquired from local markets in La Rioja and Comunitat Valenciana (Spain) between 2014 and 2015. Legumes were classified by their scientific name, colour of grain and typical production place in Spain as shown in Table 1.

Legumes were crumbled and pulverized with a domestic mill Moulinex (Barcelona, Spain) until to have an homogeneous sample. Powdered samples were stored in polyethylene bottles and preserved in desiccator till analysed. Then 0.1 g of dry homogenous sample were accurately weighed inside a nickel boat previously cleaned with a gas torch (Oklahoma, USA) and sample was moistened with 0.1 mL of ultrapure water with a minimum resistivity of 18.2 MΩ cm obtained from a Milli-Q Millipore system (Bedford, USA). The wet sample was directly introduced into the TDA-AAS system DMA-80 Millestone (Sorisole, Italy). The sample was initially dried (200 ºC, 90 s) and then thermally decomposed (650 ºC, 60 s) in a continuous flow of oxygen C-45 (200 mL min^{-1}) from Carburos Metálicos (Barcelona, Spain). Combustion products were trapped in a catalyst hot bed. Then sulphur oxides, nitrogen oxides and halogens, which can interfere the Hg analysis, were flushed out of the system and Hg (0) was selectively trapped on a gold trap. This procedure was repeated twice again, thus Hg from 0.3 g of sample was preconcentrated as a gold amalgam, and subsequently it was desorbed for absorbance measurement. The mercury content in legumes was reported as ng g^{-1} and presented as average values plus minus the standard deviation of three replicates.

Table 1. List of analyzed legume samples

Scientific name	Common name	Origin	Color
Phaseolus vulgaris	Arrocera bean	Comunitat Valenciana	White
Phaseolus vulgaris	Pinto bean	Castilla y León	Brown, brown spotted
Phaseolus vulgaris	Kidney bean	Castilla y León	Red
Vinga unguiculata	Carilla bean	Extremadura	White, black spotted
Phaseolus vulgaris	Fabes	Asturias	White
Vigna angularis	Frijoles	Euiskadi	Black
Phaseolus vulgaris	Pinto bean	Euiskadi	Brown
Lens culinaris	Castellana lentil	Castilla la Mancha	Brown
Lens culinaris	Beluga lentil	Castilla León	Black
Lens culinaris variabilis	Pardina lentil	Castilla León	Brown
Lens culinaris dupuyensis	Verdina lentil	Castilla la Mancha	Green
Phaseolus vulgaris	Black caparrón	La Rioja	Black
Phaseolus vulgaris	White caparrón	La Rioja	White, brown spotted
Phaseolus vulgaris	Pinto caparrón	La Rioja	Brown, brown spotted
Phaseolus vulgaris	Red caparrón	La Rioja	Red
Cicer arietinum	Castellano chickpea	Andalucia	Brown
Cicer arietinum	Pedrosillano chickpea	Castilla y León	Brown
Pisum sativum	Pea	La Rioja	Green
Phaseolus vulgaris	Green bean	Castilla y León	Green
Vicia faba	Broad bean	Comunitat Valenciana	Green

RESULTS AND DISCUSSION

Study of mercury measurements conditions

Preliminary studies were focussed on the amount of sample, ranging from 0.1 till 0.4 g and the evaluation of the sample thermal decomposition step. As can be seen in Figure 1, sample mass higher than 0.3 g produce overestimation of Hg concentration because thermal degradation of high amount of sample generates a lot of fumes that interfere during measurement step. So 0.1 g of sample were selected as an adequate amount and it was decided to amalgam 3 cycles to assure enough mass of Hg retained in the gold amalgam and subsequently quantitative results at low levels of Hg. The thermal decomposition time was evaluated from 60 to 180 seconds concluding that there was not a significant difference between results. So the shortest time, 60 s was selected as an adequate thermal decomposition time.

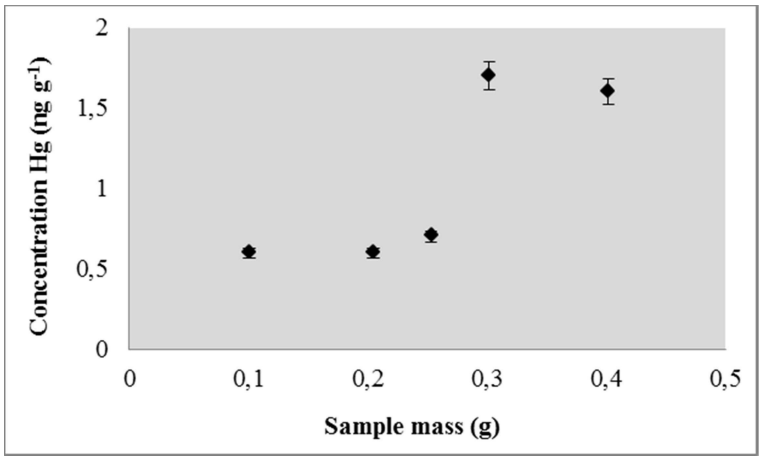

Fig. 1. Influence of sample mass on Hg measurement by TDA-AAS

Analytical characteristics of the method

The limit of detection of Hg based on the developed procedure was 0.021 ng g^{-1} in the original sample. All samples were analysed in triplicate including certified reference materials method blanks. The precision of the method, evaluated through the relative standard deviation corresponding to three measurements, varied between 3.8 and 19 % as a function of the Hg level (0.387 ng g^{-1} and 0.21 ng g^{-1}, respectively). The accuracy was verified by repeated analysis of certified reference materials through the mean percentage recovery. For Fly Ash NIST 1633b (National Institute of Standards and Technology, USA) the recovery was 95.5 ± 1.1 % and mean percentage recovery for Rice Flour NIST 1589a was 118.4 ± 1.0 %.

Analysis of samples

Total Hg concentrations have been quantified in the 20 legume samples described in Table 1. Results found are summarized in Table 2.

Total Hg was 0.171 ng g^{-1} in pea sample and ranged from 0.127 till 0.53 ng g^{-1} in beans, from 0.387 till 0.47 ng g^{-1} in chickpeas and between 0.24 and 0.30 in lentils ng g^{-1}. So, it can be concluded that all the results are in the same order of magnitude, with an average value for the 20 samples of 0.29 ± 0.11 ng g^{-1}. In comparison with data previously found in legumes from Kashmir region (Waheed et al., 2003), it can be seen that results found in Spanish legumes are two orders of magnitude lower, probably due to a contamination of the production area in Kashmir region.

Table 2. Mercury concentration in Spanish legume samples

Common name	Hg (ng g^{-1})
Arrocera bean	0.31 ± 0.05
Pinto bean	0.49 ± 0.07
Kidney bean	0.127 ± 0.009
Carilla bean	0.53 ± 0.04
Fabes	0.169 ± 0.013
Frijoles	0.168 ± 0.016
Pinto bean	0.29 ± 0.04
Castellana lentil	0.280 ± 0.011
Beluga lentil	0.25 ± 0.03
Pardina lentil	0.30 ± 0.03
Verdina lentil	0.24 ± 0.04
Black caparrón	0.303 ± 0.014
White caparrón	0.28 ± 0.03
Pinto caparrón	0.199± 0.017
Red caparrón	0.31 ± 0.04
Castellano chickpea	0.47 ± 0.03
Pedrosillano chickpea	0.387 ± 0.015
Pea	0.171 ± 0.012
Green bean	0.21 ± 0.04
Broad bean	0.36 ± 0.03

Evaluation of mercury weekly intake due to legume consume

The estimated weakly intake (EWI) was calculated by using the following equation: EWI = 7 CHg (IR/BWa); where CHg = the concentration of mercury in legume (ng g^{-1}), IR = the estimated individual ingestion rate of legumes (g day^{-1}), BWa = average body weight of adult. The intake per capita of legumes by the general population in Spain was estimated as 8.38 g day^{-1} based on the Annual Report about Food Consumption of Ministry of Agriculture, Food and Environment (MAGRAMA, 2015). The average adult body weight was estimated as 60 kg. So, estimated weekly intake of Hg varied between 0.12 to 0.52 ng kg^{-1} body weight, all far below PTWI of 4 μg kg^{-1} body weight established by FAO/WHO (JECFA, 2011), indicating not pose via consumption of the legumes analysed.

Monitoring Hg in different source of food is important to advance in the knowledge of Hg toxicity via food intake. It is well known that fish and other seafood, in aquatic environment, trend to bioaccumulate Hg and biomagnify it in the food chain; concentrations found were up to 11.4 μg g^{-1} (JECFA, 2011). However Hg in meat, vegetables, cereals and legumes could be

relevant because Hg from soils is easily uptaken by some species and that suppose another via of entrance in the food chain. From a report of Hg in meat, mercury concentration was in the range 1.9–3.5 ng g^{-1} (Ortega-Barrales and Fernández-de Córdova, 2015). Hg in mushrooms has been also well documented because mercury can be efficiently bioaccumulated by many mushrooms and show elevated concentrations at part per million order i.e up to 8.4 mg kg^{-1} (Falandysz and Borovička, 2013). On the other hand, Hg in cereals from non-contaminated areas was up to 55 ng g^{-1} in rice and wheat (Ruiz-de-Cenzano et al., 2015). Results from this study lower than 1 ng g^{-1} are comparable with low levels of mercury found in edible nuts in the 0.9 to 2.5 ng g^{-1} range in 25 samples of Brazil nuts, cashew, pistaccio, peanut, almond and walnuts (da Silva et al., 2014).

Mercury in the frame of mineral profile of legumes

In comparison with mineral profile of legumes reported in a literature revision between year 1999 and 2013 performed by Ruiz-de-Cenzano et al. (2015), data of Hg in legumes from this study, lower than 1 ng g^{-1}, were in all cases of the same order that the lowest concentration quantified on legumes that correspond to lanthanide elements (Ruiz-de-Cenzano et al., 2015). In general mineral profile for legumes produced in uncontaminated areas, run since ppb level to percentage as follows: lanthanides, As, Bi, Hg, Sb<Cd, Co, Cr, Pb, V< <Ba, Br, Mo, Ni, Cu, Se, Sr < Al, Fe, Mn, Na, Rb, Zn< Ca, Cl, Mg, P < <K as can be seen in Figure 2.

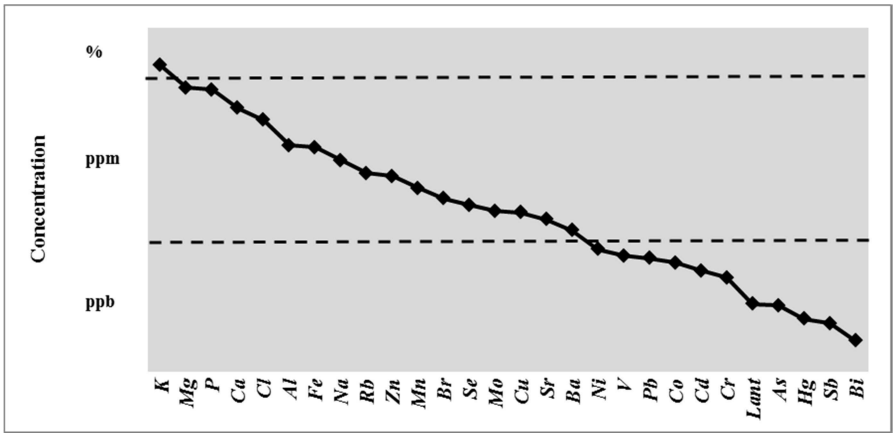

Fig 2. Mineral profile of legumes

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2.3. Especiación no cromatrográfica de mercurio en setas

Non-chromatographic speciation of mercury in mushrooms

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Non-chromatographic speciation of mercury in mushrooms

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A fast, sensitive and cheap procedure has been developed for the determination of inorganic mercury (i-Hg) and organic mercury (o-Hg) in mushroom samples. The procedure is based on the use of cold vapour atomic fluorescence spectrometry (CV-AFS). The method involves the extraction of total mercury (t-Hg) with diluted HCl, followed by measurements of the corresponding Hg vapour under two different conditions: (i) directly to determine i-Hg, and (ii) after oxidation with a mixture $KBr/KBrO_3$ to determine t-Hg. o-Hg was estimated from the difference between t-Hg and i-Hg. Previous studies were focused on the assessment of different reagents for mercury extraction and breakdown of organomercury compounds. The limit of detection values for the method were 3.2 ng g^{-1} for t-Hg and 0.6 ng g^{-1} for i-Hg, expressed in terms of sample dry weight. The mean relative standard deviation values in actual sample analysis were below 4%. The comparison of t-Hg data with results obtained through a reference direct mercury analyser evidenced the complete extraction of Hg species employing the developed method. Recovery studies provided percentages between 95 and 104% for all spiked samples, indicating that species interconversion was avoided under the selected experimental conditions. The results obtained for commercially available mushroom samples varied from 271 to 620 ng g^{-1} dry weight with 81–91% of i-Hg and 9–19% of o-Hg.

1. Introduction

Mercury is a global pollutant with a complex biogeochemistry cycle dominated by its redox state and chemical forms. The most toxic species of mercury are organic ones, especially methylmercury (MeHg), which enters organisms easily and remains for a long term causing chronic effects.[1]

Owing to its liposoluble character and high affinity for selenium, MeHg is efficiently absorbed by the gastrointestinal tract, crosses the placental and blood–brain barriers and sequesters Se. MeHg compromises selenoenzyme activities and their synthesis, thus causing devastating effects, particularly in the brain and neuroendocrine tissues.[2]

For humans, foods are the major routes of exposure to mercury. Food safety authorities have established a tolerable weekly intake (TWI) of 4 μg kg^{-1} body weight for Hg[3] and 1.3 μg kg^{-1} body weight for MeHg.[4]

The intake of mercury occurs mainly as MeHg *via* fish and seafood product consumption, but some vegetables, legumes or cereals, with relatively low mercury levels but highly consumed, can also make an important contribution.[5]

Mushrooms have become an important diet component in many countries due to their excellent flavor and nutritional value, being well known that they accumulate relatively high concentrations of mercury depending on mushroom varieties and environmental and geographic conditions.[6–10] So, monitoring the chemical forms of mercury, in addition to the determination of total mercury, in mushrooms could be important for intake risk assessment.

Analytical methods for total mercury determination in mushrooms are widely developed and have been applied to the analysis of a wide variety of mushrooms. Recently published data showed up to 22 μg g^{-1} Hg (dry weight) for mushrooms grown in unpolluted sites.[9,11–16]

Speciation of Hg requires very sophisticated methods using less aggressive extraction processes under moderate conditions of pH and temperature, and the employment of sensitive detection techniques. Procedures fully developed for Hg speciation in fish are not always useful for other samples because of different concentration levels involved, chemical forms and matrix behaviour. Therefore it is important to develop specific analytical methodologies for Hg speciation in vegetables and mushrooms. In early studies, Stegnar (1973), Stijve (1974), Minagawa (1980), Bargagli (1984) and Fischer (1995) employed gas chromatography atomic absorption spectroscopy (GC-AAS) with a limit of detection (LOD) for MeHg of about 0.01 μg g^{-1}.[17–21] Recently, Wuilloud (2004) determined the differences in the fractionation patterns of Hg through size exclusion chromatography inductively coupled plasma mass spectrometry (SEC-UV-ICP-MS)[22] and Rieder (2011) and Pilz (2011) employed GC atomic fluorescence spectroscopy

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(GC-AFS) with a LOD for MeHg of about 0.05 ng g^{-1}, and liquid chromatography (LC) combined with chemical vapour generation (CVG) and ICP-MS, respectively.[23,24] In the aforementioned studies, MeHg expressed as percentage from t-Hg ranged between 0.01 and 26%.

Non-chromatographic speciation methodologies can offer enough information in many situations, also involving a fast and simple alternative to hyphenated techniques.[25] They are based on the different behaviour of inorganic and organic mercury in the presence of reducing agents,[26] the selective retention or elution of species through a solid phase extraction system involving the use of different complexing or chelating agents,[27] or alternatively the biosorption on microorganisms (bacteria, algae, and yeast).[28,29] So, the goal of this work has been the development of a non-chromatographic procedure, which could be incorporated as a screening method into the routine analysis processes, based on soft extraction of Hg species from mushroom samples and their sequential determination by CV-AFS.

2. Experimental

2.1. Instruments and apparatus

Mushroom samples were freeze-dried using a Telstar Cryodos system (Barcelona, Spain).

Determination of mercury was carried out using a cold vapour atomic fluorescence spectrometry system PSA 10.025 Millennium Merlin instrument (Kent, UK), with a high intensity source BDHCL (boosted discharge hollow cathode lamp) superlamp from Photron (Victoria, Australia). Possible traces of mercury were removed from CV-AFS reagent solutions by using a nitrogen stream (purity higher than 97.5%) obtained from a Claind generator (Lenno, Italy). A water bath model Tectron 200 from Selecta (Barcelona, Spain) was used during sample pre-treatment.

Ultrapure water, with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q system Millipore (Bedford, USA).

2.2. Reagents and standards

All chemicals used were of the highest purity available and all solutions were prepared in ultrapure water.

Hydrochloric acid 37% from Merck (Darmstadt, Germany) was employed for Hg species extraction. A mixture 0.85 mol L^{-1} KBr/0.17 mol L^{-1} $KBrO_3$ prepared from the corresponding salts, and hydroxylamine hydrochloride 12% (w/v), both obtained from Scharlau (Barcelona, Spain), were used in sample pre-treatment. Other reagents used in the methodology developed were 2000 mg L^{-1} $K_2Cr_2O_7$ and 2000 mg L^{-1} $KMnO_4$ solutions from Panreac (Barcelona, Spain), H_2SO_4 95% from Merck, H_2O_2 35% from Scharlau and TMAH 25% aqueous solution from Fluka (Buchs, Switzerland).

To generate the mercury vapour prior to the AFS measurement the reductant agent used was 2% (w/v) $SnCl_2 \cdot 2H_2O$ in HCl. This solution was prepared by dissolving the salt from Merck in 10% (v/v) HCl and then it was purged with a stream of nitrogen for 30 min in order to eliminate possible traces of mercury. Argon C-45 (purity higher than 99.995%) supplied by Carburos Metálicos (Barcelona, Spain) was employed as the dryer and carrier gas.

1000 mg L^{-1} Hg(II) certified standard stock solution was obtained from Merck. 50 μg L^{-1} Hg(II) standard solution was prepared daily by sequential dilution and was used to prepare calibration standards from 0.2 to 1.2 μg L^{-1} in HNO_3 50% (v/v) medium.

2.3. Materials and decontamination

Taking into account the low concentration level of mercury in mushroom samples any minimum loss or contamination during sampling, storage or analysis might cause a serious systematic error in mercury determination. Therefore high care in selecting the appropriate material and its cleaning procedure must be taken.[30]

From the results found in a previous cross contamination study[31] all flasks and beakers were washed and then subjected to an additional cleaning procedure with 10% (v/v) HNO_3 for 15–30 min, and then with 10% (v/v) HCl overnight. The gas–liquid separator chamber of the CV-AFS system was also cleaned in this way daily.

2.4. Samples

Samples of a highly consumed type of mushroom (*Lactarius deliciosus*), coming from different areas across Spain, were purchased from local markets in Valencia (Spain). Fresh samples were carefully washed; the edible parts were cut and crushed, and they were frozen at −20 °C. After that, they were lyophilized for approximately 48 h at a chamber pressure of 0.05 mbar. Dried samples were crumbled and pulverized with a mill until a homogeneous mixture was obtained. Powdered samples were stored in polyethylene bottles and preserved inside a desiccator till analysis.

2.5. General procedures

2.5.1. Reference direct analysis for total mercury determination. Reference data of total Hg in mushroom samples were obtained through direct thermal degradation followed by atomic absorption analysis (TDA-AAS) using the methodology developed in the previously published work,[32] and employed for comparative purposes.

2.5.2. Speciation of mercury. Representative dried samples of 0.2 g were weighed inside pyrex glass tubes. Then, 10 mL of 4.9 mol L^{-1} HCl were added and the tubes were shaken vigorously by hand. The solid residues were filtered (70 mm Whatman No. 1 paper) and the filtrates were made up to 25 mL with ultrapure water.

Mercury speciation was performed according to a sequential oxidation strategy. For i-Hg determination, an aliquot of 10 mL of sample extract was acidified with 25 mL of concentrated HNO_3 and made up to 50 mL with ultrapure water. For t-Hg determination, another aliquot was acidified, merged with 1 mL of a mixture of KBr/$KBrO_3$ 0.85/0.17 mol L^{-1}, treated with 70 μL of 12% (w/v) hydroxylamine hydrochloride and diluted with ultrapure water. Hg was determined in both cases by CV-AFS under experimental measurement conditions indicated in Table 1 and

Table 1 CV-AFS conditions for the determination of Hg in mushrooms

Parameter	Value
Resonance wavelength (nm)	254
Measurement mode	Peak height
Gain	100
Delay time (s)	15
Analysis time (s)	40
Memory time (s)	60
Carrier flow rate (mL min^{-1})	9
Sample flow rate (mL min^{-1})	9
$SnCl_2$ flow rate (mL min^{-1})	4.5
Carrier gas flow rate (mL min^{-1})	250
Dryer gas flow rate (L min^{-1})	2.5
HNO_3 (mol L^{-1})	5
$SnCl_2$% (w/v)	2

Table 2 Recovery of total mercury from mushroom samples by applying different extraction media[a]

	t-Hg recovery[b] (%)
(a) HCl 2.4 mol L^{-1}	54 ± 4
(b) HCl 4.9 mol L^{-1}	98.5 ± 1.3
(d) HCl 3 mol L^{-1} and HNO_3 1 mol L^{-1}	Non-viable
(e) HNO_3 4.7 mol L^{-1}	40 ± 12
(f) HNO_3 1.2 mol L^{-1} and H_2O_2 2.2 mol L^{-1}	Non-viable
(g) H_2SO_4 14, HNO_3 1.2, H_2O_2 2.2 mol L^{-1}	91 ± 8
(h) TMAH 25% (w/v)	20.2 ± 1.5

[a] Reference value obtained by direct analysis TDA-AAS. [b] Results reported are the average of 2 independent analyses ± the corresponding standard deviation. Note: 0.2 g of sample were treated with the extractant reagent and resultant solution was diluted in an oxidant-acid medium prior to CV-AFS analysis.

using external calibration. An estimation of the o-Hg content was obtained from the difference between t-Hg and i-Hg.

3. Results and discussion

3.1. Stability of the extracted samples

One of the most important parameters which can affect the Hg stability in dissolved samples is the container material used during sample storage. Several possible mechanisms have been suggested to explain the loss of mercury including adsorption on the container walls and volatilization.[30] So, in order to evaluate Hg losses during storage, the extracts of mushroom samples were introduced inside pyrex and polyethylene containers and were analysed after different days of storage. The results found (see Fig. 1) evidence that pyrex containers are suitable to store the solutions for a period of three days whereas polyethylene containers are strongly advised against.

3.2. Evaluation of sequential extraction of mercury species

Previous studies (see Table 2) were carried out to investigate mercury species extraction capability by using the two main methods of sample leaching described in the literature: alkaline extraction and acid leaching.[33–35] To do it 0.2 g of sample were treated with different concentrations of HCl, HNO_3, or mixtures of two or three reagents between HCl, HNO_3, H_2O_2 and H_2SO_4, with TMAH also being assayed as the extractant. The use of HNO_3 in combination with H_2O_2 or HCl is not useful because the strong vapours generated led to leaks of Hg. Treatment with TMAH gave the poorest results probably because of the need for an extra amount of acid to compensate the basic medium for the generation of Hg(0) in the CV-AFS system. A diluted mixture of H_2SO_4, HNO_3 and H_2O_2, successfully employed in the speciation of Hg in fish by Cava *et al.* (2004),[26] provided good results with 91% recovery. The single use of HNO_3 4.7 mol L^{-1} or HCl 2.4 mol L^{-1} did not give quantitative results. However HCl 4.9 mol L^{-1} provided the best results with 98.5% recovery.

On the other hand, the use of an extra energy for the extraction process was considered. Ultrasound energy, which is generally recommended to accelerate the leaching of mineral elements from solids, in this case leads to losses of mercury (see Fig. 2). In contrast, good accomplishment was obtained by shaking pyrex tubes mechanically or by hand.

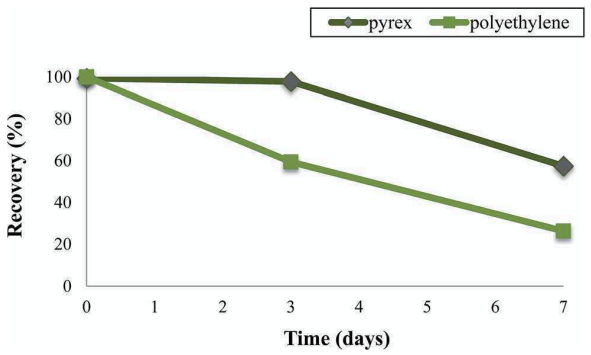

Fig. 1 Effect of the container material on the stability of mercury in acid extracts during sample storage.

3.3. Degradation of organomercurials

The determination of organic mercury by CV-AFS needs a previous degradation by oxidation to inorganic Hg(II). Common reagents used for this purpose like $K_2Cr_2O_7$, $KMnO_4$ and $KBr/KBrO_3$ were tested for the analysis of MeHg from standard solutions. Table 3 shows that a small amount of $K_2Cr_2O_7$ or $KMnO_4$ does not break quantitatively the organic bond, and furthermore provided a very low MeHg recovery. Additional studies with $KBr/KBrO_3$ were done to investigate the effect of the oxidant concentration at room temperature and at 50 °C (see Fig. 3). The best recovery percentages were obtained with a concentration of 0.85 mol L^{-1} KBr and 0.17 mol L^{-1} $KBrO_3$ and heating the reaction mixture at 50 °C in a water bath for 15 minutes.

3.4. Analytical characteristics

The sensitivity of the proposed method was evaluated from the calibration slopes. The limit of detection LOD was established

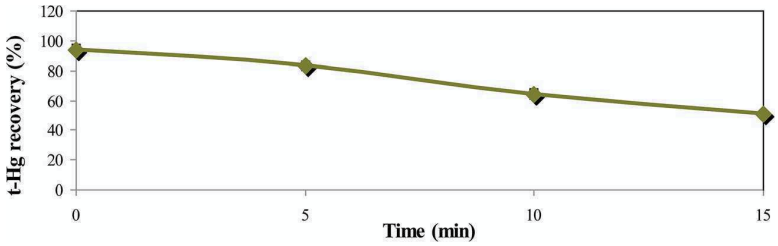

Fig. 2 Effect of the sonication time used in the extraction procedure on total mercury recovery from a mushroom sample when it was treated with 4.9 mol L^{-1} HCl and the resultant solution was diluted in an oxidant-acid medium prior to CV-AFS analysis. The results reported are the average of 2 independent analyses ± the corresponding standard deviation.

Table 3 Capacity of several reagents for organomercurial breakdown[a]

	MeHg recovery (%)
(a) $KMnO_4$ 2 mg L^{-1}	16
(b) $K_2Cr_2O_7$ 4 mg L^{-1}	44
(c) $KBr/KBrO_3$ 0.085/0.017 mol L^{-1}	60

[a] Note: 1 ng mL^{-1} MeHg standard was employed as the test sample.

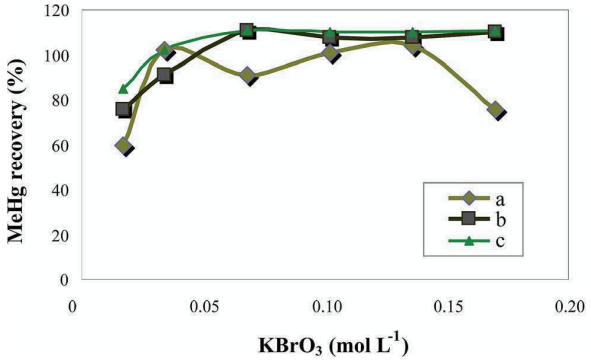

Fig. 3 Effect of $KBrO_3$/KBr concentration on recovery of MeHg by treatment of a standard solution, (a) room temperature, (b) 50 °C during 15 minutes, (c) 50 °C during 30 minutes. Note: 1 ng mL^{-1} MeHg standard was employed as test sample; the reagent concentrations $KBrO_3$/KBr were always maintained in 1 : 5 proportion.

using the 3 s criterion and the limit of quantification LOQ was based on the use of the 10 s criterion (see Table 4), and it can be concluded that the sensitivity of the method is higher than those found by other authors employing GC-AAS methods[17–20] and little bit higher than those found by GC-AFS.[23,24] The precision of the procedure was evaluated as the relative standard deviation RSD% for 8 replicate analyses of a natural sample, with obtained RSD values of 3.2 and 2.5% for t-Hg and i-Hg determinations.

To check the possibility of losses, contamination or species interconversion during the whole procedures, mushroom samples were spiked prior to digestion or extraction at 400 ng g^{-1} level with i-Hg and at 80 ng g^{-1} level with o-Hg and recovery values were determined. The aforementioned data are

Table 4 Analytical characteristics of the CV-AFS determination of Hg in mushrooms

	t-Hg	i-Hg
Sensitivity/fluorescence (ng^{-1} mL)	330–370	410–450
Correlation coefficient	0.9998	0.9997
LOD (ng g^{-1})[a]	3.2	0.6
LOQ (ng g^{-1})[a]	10.5	1.9
RSD (%) ($n = 8$)[b]	3.2	2.5
Recovery (%), 400 ng g^{-1} Hg^{2+} added[c]	99 ± 2	95 ± 7
Recovery (%), 80 ng g^{-1} $MeHg^{+}$ added[c]	104 ± 13	6 ± 2

[a] Limits of detection, LOD and limits of quantification, LOQ were calculated as the concentrations corresponding to signals equal to three-times or ten-times, respectively, the standard deviation of ten blank solutions and taken into consideration the amount of samples extracted and the dilution of samples made after extraction. [b] Relative standard deviation, RSD were calculated in percentage from the average of the repeatability of eight independent analyses made on actual samples. [c] Recovery studies were made for actual mushroom samples spiked with a known concentration of Hg^{2+} and $MeHg^{+}$. Results are the average ± standard deviation of 3 independent analyses.

presented in Table 4, and it was confirmed that the quantitative recovery of MeHg during total Hg determination and the relative low recovery of 6 ± 2% when direct i-Hg determination was carried out.

Regarding green analytical parameters, an assessment of reagent toxicity, energy inputs, occupational hazards and toxic waste has been done according to the use of the Analytical Eco-Scale first described by Galuszka *et al.* (2012).[36]

Toxic waste management can be done with deactivation and minimization of heavy metals through a treatment based on co-precipitation with iron in an alkaline medium. It was concluded that the developed methodology for Hg speciation in mushrooms suppose an acceptable green analysis.[32]

3.5. Analysis of samples

Table 5 summarises concentration data obtained for eight mushroom samples of the *Lactarius deliciosus* species. Total mercury obtained through selective determination by CV-AFS ranged from 271 to 620 ng g^{-1} dry matter. There is a clear correlation between data found by the aforementioned procedure (y) and data obtained by the reference method of TDA-AAS (x), using the correlation equation $y = (0.93 \pm 0.03)x + (10 \pm 14)$,

Table 5 Analysis of mushroom samples from Spain[a]

	Reference TDA-AAS	Selective determination CV-AFS		
Sample	t-Hg	t-Hg	i-Hg	o-Hg
M1	441 ± 9	403 ± 12	320 ± 30 (82%)	74 ± 19 (18%)
M2	487 ± 13	450 ± 20	365 ± 19 (81%)	90 ± 40 (19%)
M3	649 ± 18	620 ± 20	530 ± 50 (85%)	90 ± 60 (15%)
M4	280 ± 20	271 ± 11	246 ± 12 (91%)	25 ± 2 (9%)
M5	340 ± 30	333 ± 5	294 ± 5 (88%)	39 ± 10 (12%)
M6	570 ± 20	538 ± 17	470 ± 30 (87%)	70 ± 30 (13%)
M7	320 ± 30	310 ± 3	261 ± 14 (84%)	49 ± 14 (16%)
M8	416 ± 5	408 ± 5	342 ± 16 (84%)	66 ± 14 (16%)
M9	357 ± 7	326 ± 4	290 ± 9 (92%)	35 ± 12 (11%)

[a] Concentration expressed in ng g^{-1} referred to dried sample. Results reported are the average of 3 independent analyses ± the corresponding standard deviation. Values in parenthesis are the percentage of each species with respect to the total mercury content.

with $R^2 = 0.992$. Experimental Student's *t*-values of 2.29 and 0.49 were obtained for the slope and the intercept, respectively, thus, indicating in both cases that a slope of 1 and an intercept of 0 were statistically found at a probability level of 95% because the experimental *t* values were lower than the theoretical one, which was 2.45. So, it can be concluded that both employed methods have a comparable accuracy.

Organic mercury data found from this study ranged between 25 and 90 ng g^{-1} dry weight, which stands from 9 till 19% of total Hg concentrations.

3.6. Comparison of data with literature studies on mercury speciation in mushrooms

Organic mercury data obtained by applying the developed methodology were in very good agreement with data previously reported in the literature for MeHg obtained by chromatography-based speciation methodologies. Stijve and Roschnik (1974) studied the methylated mercury content in wildlife samples from a non-polluted area and found values between 1 and 26%.[18] Minagawa *et al.* (1980) and Fischer *et al.* (1995), which focused their studies on mushrooms from a forest near an acetaldeyde factory in Japan and mushrooms from a mining area in Germany, found similar results between 2.9 and 9.1% and from 0.4 to 19% of MeHg, respectively.[19,21] Bargagli and Baldi (1984) found low amounts, below 3.7% of MeHg with respect to t-Hg, in mushrooms from a mining area in Italy.[20] Recently Rieder *et al.* (2011) reported contents of the methylated form between 0.3 and 11.3% of the total Hg in different mushroom species from non-contaminated forest soils in Switzerland.[23]

4. Conclusions

A fast, non-chromatographic method has been developed and validated to determine inorganic and organic mercury species in mushrooms. From the studies carried out it can be concluded that the method provides good recoveries and comparable results with those obtained from a reference procedure and those reported in the literature by chromatographic approaches. The analysis of *Lactarius deliciosus* mushrooms from different geographic origins in Spain, reveals a total mercury content up to 620 μg kg^{-1} (dry weight) with the moisture content in mushrooms around 90%. The highly toxic organic mercury was a minor fraction (<20%) of the total Hg in mushrooms. These results evidenced that a normal consumption of mushrooms will not result in exceeding the limits for the tolerable weekly intake of total mercury and methylmercury. So, the mushroom consumption does not imply any health risks for the people in Spain.

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2.4. Especiación no cromatrográfica de metilmercurio en pescado

Speciation of methylmercury in market seafood by thermal degradation, amalgamation and atomic absorption spectroscopy

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Speciation of methylmercury in market seafood by thermal degradation, amalgamation and atomic absorption spectroscopy

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ABSTRACT

Sample thermal decomposition followed by mercury amalgamation and atomic absorption has been employed for the determination of methylmercury (MeHg) in fish. The method involves HBr leaching of MeHg, extraction into toluene, and back-extraction into an aqueous L-cysteine solution. Preliminary studies were focused on the extraction efficiency, losses, contaminations, and species interconversion prevention. The limit of detection was 0.018 $\mu g\,g^{-1}$ (dry weight). The intraday precision for three replicate analysis at a concentration of 4.2 $\mu g\,g^{-1}$ (dry weight) was 3.5 percent, similar to the interday precision according to analysis of variance (ANOVA). The accuracy was guaranteed by the use of fortified samples involving 83–105 percent recoveries, and certified reference materials TORT-2 (lobster hepatopancreas) and DORM-3 (dogfish liver), providing 107 and 98 percent recovery of certified values. The greenness of the method was also evaluated with the analytical eco-scale being obtained a final score of 73 points which means an acceptable green analysis. The method was applied to fifty-seven market samples of different fish acquired from local markets in several sampling campaigns. The content of MeHg found varied between 0.0311 and 1.24 $\mu g\,g^{-1}$ (wet weight), with values that involve 33–129 percent of the total mercury content. Some considerations about food safety were also done taking into account data about Spanish fish consume and Tolerable Weekly Intake (TWI) established for MeHg.

1. Introduction

Methylmercury (MeHg) is considered the most toxic mercury species due to its bioaccumulative character and biomagnification through the food chain (Driscoll et al., 2013). It is efficiently absorbed from the gastrointestinal tract and it is able to cross the placenta and the blood-brain barrier (Gundacker et al., 2010), thus creating neurological disorders, especially in foetus and children. Unfortunately, human intake of mercury occurs mainly as MeHg via fish consumption (European Food Safety Authority (EFSA), 2012).

The European Food Safety Authority (EFSA) recommended in 2012 a Tolerable Weekly intake (TWI) for MeHg of 1.3 $\mu g\,kg^{-1}$ body weight (bw), expressed as mercury, which provides a margin of about 40 as compared to the Lower Benchmark Dose on the 95 percent confidence level ($BMDL_{05}$) for the reduction in antibody response in rats (European Food Safety Authority (EFSA), 2012).

In the European Union, Maximum Tolerated Level (ML) for mercury in fish is 0.5 mg kg^{-1} wet weight (ww) or 1.0 mg kg^{-1} ww (predatory fishes) (European Comission (EC), 2008). However, the best way of managing the risk of MeHg exposure is the combination of appropriate MLs and consumer advice, because patterns of fish consumption and mercury level in fish vary between countries. Hence, it is important to develop, maintain, and improve existing databases on MeHg focused on regional or national-based information (Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), 2013).

The most commonly methods for MeHg determination involve the use of sophisticated hyphenated techniques with gas chromatography or liquid chromatography coupled to highly selective and sensitive detectors; such as atomic absorption spectroscopy (Jagtap et al., 2011), atomic fluorescence spectroscopy (Ohki et al., 2013), atomic emission spectroscopy, and inductively coupled plasma mass spectrometry (Tu et al., 2000).

Non-chromotagraphic methods employing cold vapour techniques, often in combination with preconcentration procedures, have been also developed as a simpler and cheaper alternative for MeHg determinations. They are based on the different behaviour of inorganic and organic mercury in front of reducing agents (Cava-Montesinos et

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al., 2004) (Ashkenani et al., 2009), the selective retention or elution of species through a solid phase extraction system involving the use of different complexing or chelating agents (Vereda Alonso et al., 2008) (Wu et al., 2006), or alternatively the biosorption on microorganisms (bacteria, algae, yeast) (Tuzen et al., 2009a,b).

In recent years, the occurrence of direct mercury analyser instrumentation based on thermal degradation, amalgamation and atomic absorption (TDA-AAS) has improved the determination of total mercury at trace levels, because it allows an accurate quantification at very low concentrations in a very fast way. However, the use of this technique in mercury speciation requires a sample pre-treatment in order to separate the target species prior to the measurements. Some approaches have been published involving acid extraction of MeHg with HCl or HBr and double liquid–liquid extraction, first with toluene and then with cysteine or tiosulphate solution (see Table 1).

However, many of the available studies remain at the academical level or have been applied to a reduced number of samples or fish species. In line with the European Commission Joint Research Centre Institute for Reference Materials (EC-JRC-IRMM) which has recently run a collaborative trial in an attempt of standardise a method for MeHg determination (European Commission Joint Research Centre Institute for Reference Materials and Measurements (EC-JRC-IRMM), 2013), studies performed on certified reference materials should be now extended to field studies in order to substantiate the applicability of the available methodologies.

The purposes of this work were to assess the suitability of TDA-AAS methodology for MeHg determination in natural fish samples, also evaluating the greenness of the method, and to improve the existing databases regarding MeHg in fish consumed by the Spanish population.

2. Material and methods

2.1. Apparatus and instruments

Freeze-dry process was performed with a Telstar Cryodos system (Barcelona, Spain). Sample preparation involved the use of an analytical balance Mettler Toledo (Barcelona, Spain), a centrifuge Ortoalresa Mod Digicen 20 (Madrid, Spain) and a Milli-Q system Millipore (Bedford, USA) for ultrapure water. Mercury determination based on sample thermal degradation atomic absorption spectroscopy was made using a dual cell spectrophotometer Milestone instrument DMA-80 (Sorisole, Italy).

2.2. Reagents, standards and certified samples

All chemicals used were of the highest purity available and all solutions were prepared in ultrapure water with a resistivity 18.2 MΩ cm. HNO_3 69 percent (w/v) from Scharlau (Barcelona, Spain) was employed for cleaning glassware materials. In sample treatment, HBr 47 percent (w/v) and toluene, both from Scharlau, and L-cysteine solution from Sigma (Barcelona, Spain) were used. Oxygen N-50 was supplied by Carburos Metálicos (Barcelona, Spain).

Certified standard stock solution of 1000 mg L^{-1} Hg(II) was obtained from Merck (Darmstadt, Germany) and MeHg(II) chloride was obtained from Sigma-Aldrich (Madrid, Spain).

Certified reference materials TORT-2 (lobster hepatopancreas) and DORM-3 (dogfish liver) were obtained from the National Research Council of Canada (NRC) and Coal fly ash 1633c was provided by the National Institute of Standards and Technology of USA (NIST).

Table 1
Methods proposed in the literature for the determination of MeHg in fish by TDA-AAS.

Sample treatment	Type of sample	Origin	Accuracy (%)	Reference
Ultrasound extraction with HCl(15 mL)/toluene(5 mL), back-extraction into cysteine acetate(1 mL)	BCR 463,cod, flounder, turbot, perch, herring	Poland	(90.3–102)	(Kwasniak et al., 2012)
Microwave extraction with HCl(0.75 mL)/H_2O(1 mL)/ toluene(10 mL), back-extraction into cysteine acetate (2 mL) Acid leaching with H_2SO_4/KBr(5 mL)/ $CuSO_4$(1 mL), o-Hg extraction into toluene(5 mL, 3 times) and back-extraction into $Na_2S_2O_3$(5 mL)	BCR 463, sardine, anchovy, tuna fish	Spain	(92)	(Carbonell et al., 2009)
	BCR 463, TORT-2, *D. labrax*	Portugal	(97–105) (91–107)	(Válega et al., 2006)
	S. plana	Portugal		(Ahmad et al., 2012)
	S. canicula	Atlantic Ocean		(Coelho et al., 2010)
	TORT-2, *D.labrax*	Portugal	(75–91)	(Mieiro et al., 2011)
	TORT-2, *Liza aurata*	Portugal	(89.6 ± 4.58)	(Tavares et al., 2011)
Hydrolysis with HBr(10 mL), o-Hg extraction into toluene(20 mL) and back-extraction into cysteine (6 mL)	DORM-2, DOLT-3, SRM-2976, SRM-2977, *T. bernacchii, T. pennelli, G. acuticeps, C. hatus, C. mawsoni*	Antarctic	(84.3) (73.9) (92.8) (81.2)	(Maggi et al., 2009)
	DORM-2, DORM-3, TORT-2			(Scerbo and Barghigiani, 1998)
	DORM-2, *Lophius spp., Helicolenus dactylopterus, Aphanopus carbo, Lepidorhombus spp.*	Portugal	(94.63)	(Afonso et al., 2008)
	DORM-2, TORT-2, *Prionace glauca, Xiphias gladius*	Atlantic Ocean	(100) (93.42)	(Branco et al., 2004)
Hydrolysis with HBr(10 mL), o-Hg extraction into toluene(35 mL) and back-extraction into cysteine (6 mL)	DOLT-4, TORT-2, SRM 2974a, SRM 1566b, ERM CE464		(84.6 ± 33.6) (96.4 ± 21.0) (100.9 ± 29.0) (143.1 ± 71.4) (87.3 ± 15.2)	(European Commission Joint Research Centre Institute for Reference Materials and Measurements (EC-JRC-IRMM), 2013)
Tris–HCl buffer (with sequential additions of protease, NaOH, cysteine, $CuSO_4$, acidic NaBr) followed by extraction with toluene(0.5 mL) and $Na_2S_2O_3$(0.15 mL)	DOLT-3, TORT-2, NIST 1566 b		(98.6 ± 5.7) (97.9 ± 4.7) (97.2 ± 9.6)	(Nam and Basu, 2011)

BCR 463: tuna fish. DORM-2: dogfish muscle. DORM-3: dogfish liver. DOLT-3, DOLT-4: dogfish liver. ERM CE464: tuna fish. NIST 1566 b: oyster tissue. SRM-2974a, 2976, 2977: mussel tissue. SRM-1566b: oyster tissue. TORT-2: lobster hepatopancreas.

2.3. Experimental procedures

2.3.1. Sample collection, transport and storage

Fish species studied were cod (*gadus morhua*), four-spot megrim (*lepidorhombus boscii*), gilt-head bream (*sparus aurata*), goatfish (*mullus barbatus*), hake (*merluccius merluccius*), halibut (*hippoglossus hippoglossus*), mackerel and canned mackerel (*scomber scombrus*), monkfish (*lophius piscatorius*), nile perch (*lates niloticus*), canned octopus (*octopus vulgaris*), panga (*pangasianodon hypophthalmus*), poor cod (*trisopterus minutes*), sea bream (*pagellus bogaraveo*), sole (*solea solea*), swordfish (*xiphias gladius*), thornback ray (*raja clavata*), tuna (*thunnus thynnus*) and canned tuna (*thunnus alalunga*).

Samples were purchased from different local markets in Valencia (Spain). Every studied specimen was acquired in triplicate at different campaign to guarantee a representative sampling heterogeneity. In the laboratory, fish were eviscerated and crushed with a domestic mixer Braun (Kronberg, Germany) and finally they were freeze-dried for approximately 48 h at a chamber pressure of 0.05 mbar. Dried samples were crumbled and pulverised until to have a homogeneous mixture. Powdered samples were stored in polyethylene bottles and preserved in desiccators till to be analysed (García et al., 2013).

2.3.2. Methylmercury extraction and TDA-AAS measurement

A representative dried sample of 0.2 g was weighed inside a polypropylene tube and reconstituted with 0.5 mL of water. Digestion was carry out at room temperature with 5 mL HBr and MeHg was extracted into 10 mL of toluene. Physical separation of organic and aqueous phase was performed by centrifugation at 1330 g for 10 min and let to stand overnight. A second extraction was performed with 10 mL toluene and the mixture was centrifuged in the aforementioned conditions. Both organic extracts (a total volume of 16 mL) were sequentially added to 6 mL of a 0.7 percent (w/v) L-cysteine solution. So, MeHg was back-extracted and 0.5 mL of the aqueous phase were analysed by using the TDA-AAS system.

The sample extract was initially dried and thermally decomposed in a continuous flow of oxygen. Combustion products were trapped in a catalyst hot bed. Sulphur oxides, nitrogen oxides and halogens, which can interfere with the mercury analysis, were flushed out of the system and Hg (0) was selectively trapped on a gold amalgamator and subsequently desorbed for absorbance measurement. The operation programme includes: step 1, sample drying (250 °C, 60 s); step 2, thermal degradation (650 °C, 150 s); step 3, purge (60 s); step 4, Hg desorption (12 s) and absorbance measurement at 254 nm (30 s).

Mercury concentration was determined using external calibration. Two different calibration curves were used to work with the two cells, a first cell for low levels (1–20 ng) and a second cell for high concentrations (20–1000 ng). The instrument calibration was stable during a long period of time thus making unnecessary to calibrate each day. However, the calibration plot was verified every season of work by the analysis of a certified reference sample NIST Coal fly ash 1633c.

Quality assurance was provided by the measurement of NIST Coal fly ash 1633c every 10 independent samples. Additionally each sample was prepared and measured in triplicate and two blank solutions were subjected to the same treatment for every run of samples and analysed together with treated fish specimens.

2.4. Experimental design and data analysis

The type of design employed for assessing the suitable volume of reagents employed in the general procedure was performed with Minitab®, and data were analysed by ANOVA and *F*-tests using MS Excel® software.

The variables optimised for sample treatment were HBr and toluene volume. The minimum and the maximum values of the variables were 5 and 10 mL for HBr volume and 10 and 20 mL for toluene. The response function was evaluated using the Pareto charts, lengths of the bars reflect the contribution of each effect together with their relative significance on MeHg concentration using Student's *t* statistics.

3. Results and discussion

3.1. Cleaning of materials

The volatile nature of mercury and its low concentration level in fish samples requires a high care in selecting the appropriate material and its cleaning procedure; otherwise any losses or contamination might cause serious systematic errors in the determination (Yu and Yan, 2003).

Glassware materials were systematically cleaned with 10 percent HNO_3 (v/v). However, polypropylene tubes and caps, which are in contact with the sample along the whole procedure were also investigated to get the most appropriate decontamination system. So alternatively: (i) new tubes without any treatment, (ii) new tubes let to stand for a minimum of 8 h with 10 percent HNO_3 (v/v) in a covered container or, (iii) new tubes let to stand for a minimum of 8 h with 10 percent HNO_3 (v/v) followed by 2 h with 10 percent HCl (v/v) in a covered container, were tested.

The tubes and caps were rinsed three times with ultrapure water, and blank samples were prepared and finally measured, in triplicate, in the TDA-AAS instrument. Hg signals corresponding to (i) untreated material provided 0.3, 0.5, 0.3 ng of Hg; treatment (ii) 0.8, 0.4, 0.4 ng of Hg and treatment (iii) 0.4, 0.4, 0.3 ng of Hg. These data were under the instrument absolute quantification limit (0.9 ng) in all the cases. So, untreated tubes were employed for the rest of experiments as the simplest choice.

3.2. Extraction and separation of MeHg

The systematic evaluation of HBr and toluene volume influence on single extraction efficiency has been performed using a Design Of Experiments (DOE) (Telford, 2007) provided by Minitab® Statistical Software (Fig. 1). On the Pareto charts, lengths of the bars were used to reflect the contribution of each effect together with their relative significance on MeHg concentration, established using Student's *t* statistic critical value located at $\alpha=0.05$ (2.776).

As can be seen in Fig. 1, volumes of HBr and toluene did not affect MeHg extraction from TORT-2 and DORM-3 certified reference materials significantly. So a minimum amount of 5 mL HBr and 10 mL toluene were selected for following experiments.

A second extraction process with 10 mL toluene was investigated to improve the yield of MeHg extraction. Table 2 shows results from the analysis of TORT-2, DORM-3 and tuna samples involving 1 and 2 extractions steps. As can be seen, the use of a single extraction provided a recovery of less than 90 percent of MeHg while the use of two extractions steps provided quantitative data around 100 percent.

In short, the amount of acid and toluene has been drastically reduce in comparison with the methodology described by Scerbo and Barghigiani (1998) and employed by several authors (Table 1).

On the other hand, in back-extraction step, the original procedure (Scerbo and Barghigiani, 1998) involved the use of a 1 percent (w/v) L-cysteine solution that was prepared in 12.5 percent (w/v) anhydrous sodium sulphate and 0.08 percent (w/v) sodium acetate. In these conditions, ash residues from pyrolysis were considerable and the cleaning of the instrument was frequently necessary. The requirement of salts addition was assessed by the analysis in triplicate of one sample prepared alternatively in L-cysteine or L-cysteine/anhydrous sodium sulphate/sodium acetate. Results obtained by the treatment with L-cysteine provided 1.43 ± 0.06 μg g^{-1} for a gilt-head bream sample, being obtained by the use of L-cysteine plus salts, 1.3 ± 0.3 μg g^{-1}. So, no statistical difference was found between both data sets and, because of that, the use of anhydrous sodium sulphate and sodium acetate was omitted in the following studies.

3.3. MeHg extracts stability

In the JRC study (European Commission Joint Research Centre Institute for Reference Materials and Measurements (EC-JRC-IRMM), 2013) it was verified that the sample extract was stable one week in the fridge, but the removal of anhydrous sodium sulphate and sodium acetate from cysteine solution probably could reduce the stability of the extracts. Because of that, the stability was assessed by the triplicate analysis of one extract stored for (i) 0 h, (ii) 24 h, and (iii) 48 h at ambient conditions. The mean results for these extracts were 4.92 ± 0.10; 4.87 ± 0.06; and 3.7 ± 0.8 for the three periods of time considered. So, it can be concluded that MeHg concentrations highly decreased on the third

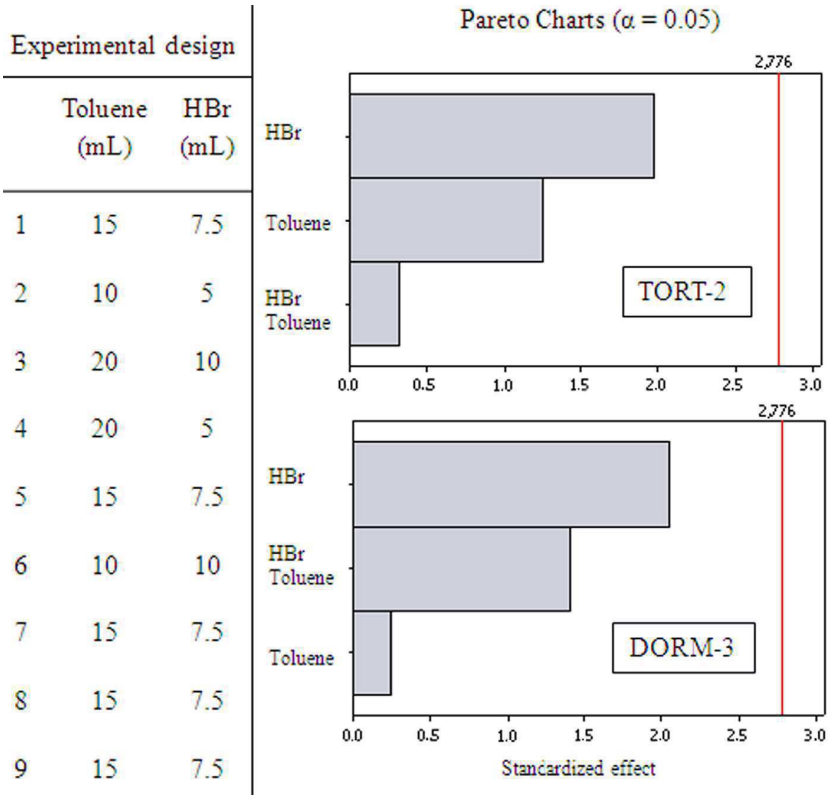

Fig. 1. Effect of HBr and toluene volume on the extractions of MeHg from fish samples.

Table 2
MeHg recovery as a function of the number of successive extractions.

C_{CRM} (μg g^{-1})	C_m (μg g^{-1})	Recovery (%)	Statistical analysis		
			Δ_m	$U\Delta$	Conclusion
TORT-2					
0.152±0.013					
1st extraction	0.134±0.005	88±3	0.018	0.014	Not comparable
1st+2nd extraction	0.163±0.013	107±9	0.011	0.02	Comparable
DORM-3					
0.355±0.056					
1st extraction	0.290±0.019	82±5	0.065	0.060	Not comparable
1st+2nd extraction	0.349±0.009	98±2	0.006	0.057	Comparable
Tuna					
1st extraction	3.3±0.4				
1st+2nd extraction	4.3±0.4				

C_m: Mean (± standard deviation) corresponding to three independent analysis performed following the general procedure, either with one extraction,10 mL toluene, either with two successive extractions with a final volume of 20 mL toluene.
C_{CRM}: Mean (±standard deviation) of certified value.
Δ_m: Absolute difference between mean measured value and certified value.
$U\Delta$: Expanded uncertainty of difference between result and certified value calculated as $U\Delta=2\times((sd/n^{1/2})^2+(U_{CRM}/2)^2)^{1/2}$.
sd: standard deviation. n: number of replicates. U_{CRM}: expanded uncertainty of each certified value given on the certificate.

day; therefore samples can be maintained at ambient conditions for up to 24 h in extraction solutions prepared with L-cysteine without adversely affecting the accurate quantification of MeHg.

3.4. Analytical response of MeHg in L-cysteine extracts

Due to the highly stable response of elemental mercury analyser, there is no need to recalibrate for each run of samples. Calibration is usually stable for at least one year. However it is mandatory to assure that the analytical response of Hg species in L-cysteine extract is comparable to the analytical response of Hg standards in aqueous medium used for instrument calibration. So, standard solutions in L-cysteine at low (150 ng mL^{-1}) and high concentration level (1000 ng mL^{-1}) were compared with measurements made in aqueous medium. Relative errors were 2.1 percent and 1.1 percent, respectively. So it was concluded that cysteine did not adversely affect the accurate quantification of MeHg.

Losses of mercury or carryover during the analysis were investigated by continuous re-analysis (six replicates) of a certified sample extract (TORT-2) placed on adjacent autosampler positions.

No differences were found between values with a relative standard deviation (RSD) of 4.4 percent. So we can conclude that automation of measurements did not affect the accurate quantification of MeHg.

3.5. Analytical characteristics of the method

Two calibration ranges, using a long cell (high sensitivity) and a short cell, were employed for Hg determination, being obtained the equations: $y=0.00088x^2+0.056x+0.0019$, $R^2=0.9998$ and $y=-7\times10^{-8}x^2+0.0007x+0.006$, $R^2=0.9996$, respectively.

The detection limit (LOD), defined as three times the standard deviation of ten blank measurements, was 0.3 ng MeHg which corresponded to 0.018 μg g^{-1} for a sample mass of 0.2 g of dry fish. The limit of quantification (LOQ), defined as ten times the standard deviation of ten blank measurements, was 0.9 ng MeHg which corresponded to 0.06 μg g^{-1} for 0.2 g of dry sample mass. Similar characteristics were obtained in other studies; such as those of Maggi et al. (2009) and Branco et al. (2004) that referred LODs of 0.4 ng, and 0.053 μg g^{-1} (dry weight) respectively. However the LOD was better than that reported by Afonso et al. (2008) which was 0.5 μg g^{-1} (dry weight).

The method precision was determined by performing three independent analyses per day (intraday precision) over three consecutive days (interday precision). Concentration values (μg g^{-1}) found were 4.12, 4.20 and 4.05 (day 1); 4.06, 4.20 and 4.05 (day 2); and 4.41, 4.42 and 4.08 (day 3). The intraday precision, employed as repeatability indicator, was assessed by RSD values every day, 1.8, 2.0 and 4.5 respectively, which were totally acceptable considering that MeHg content in the analysed sample was at μg g^{-1} level. In order to know if the day of analysis introduces an error source different to that present in the repeatability study, a one-way analysis of variance (ANOVA) was conducted with MS Excel® software. Fischer–Snedecor test was employed for variance comparison. Null hypothesis considered that intraday and interday variances did not differ statistically; and statistical *F*-calculated (2.2) was compared with *F*-tabulated (5.1) at 0.05 significance level and 2, 6 degrees of freedom (*F*-tabulated (0.05, $h-1$, h($n-1$)); h=days; n=replicate). Since *F*-calculated was lower than *F*-tabulated, the null hypothesis was accepted (Miller and Miller, 2002) The RSD of all determinations was 3.5 percent.

Accuracy was established by the analysis of certified reference materials. Recovery values of MeHg were 98 and 107 percent for DORM-3 and TORT-2 respectively. These results are in good agreement with other studies (see Table 1). With the aim of assessing the goodness of these results, a structured and quantitative approach was used taking into account the certified value (C_{CRM}), the measured result (C_m) and their respective uncertainties. These uncertainties were subsequently combined and the expanded uncertainty ($U\Delta$) was compared with the subtraction of concentrations (Δ_m). If Δ_m is lower than $U\Delta$ it can be indicated that there is no significant difference between the obtained values for CRMs and the certified ones (see Table 2) (European Comission Joint Research Centre Institute for Reference Materials and Measurements (EC-JRC-IRMM), 2008).

In addition, to evaluate the behaviour and suitability of the MeHg speciation methodology for commercial samples, several natural samples with different content in fat (tuna, gilt-head bream and sole) were spiked with MeHg and inorganic Hg standards. Recoveries of 2 μg g^{-1} MeHg fortification level ranged from 83 to 105 percent, while the presence of 1 μg g^{-1} Hg (II) spiked not increased the values of found MeHg; thus indicating that there was not mercury species interconversion during the whole process (see Table 3). It should be also notice that the lowest recovery corresponded to gilt-head bream, could be explained by the high lipid content of these samples which obstructs the liquid–liquid separation as can be observed experimentally.

3.6. Green features of the method

The aforementioned reduction in the use of reagents involves an improvement of the green features of the method. A metric approach has been employed to quantitatively evaluate the greenness of the proposed procedure. It has been used the eco-scale proposed by Van Aken and developed by Galuszka et al., (2012) which has been also employed to evaluate the TDA-AAS method in front of the atomic fluorescence spectroscopy for the determination of total Hg in mushrooms (Ruiz-de-Cenzano et al., 2014). In this study, the energy consumed, together with the amounts of reagents employed and their toxicity were evaluated, also considering the amount of wastes generated.

From an initial scale of 100 points, the use of HBr for the treatment of samples and the extraction with toluene involves 21 points penalty; the energy consume of freeze-drying instrument, furnace and spectrometer, provides an additional penalty of two points. Additional points from occupational hazard and wastes involve four penalty points. So the final score of the method was 73 points that means an acceptable green analysis.

3.7. Analysis of fish samples

Table 4 summarises the MeHg content found in this study and the percentage value respect to the total Hg content determined by García et al. (2013). The analysed fish provided heterogeneous MeHg contents which ranged from 0.0311 to 1.24 μg g^{-1} expressed in terms of sample fresh weight. The concentrations of MeHg in panga and canned mackerel of sampling campaign 2 were below the quantification limit and the highest MeHg concentrations were observed in tuna, swordfish and goatfish probably resulting from biomagnifications within predatory species. Several samples of each species were analysed in order to compensate the heterogeneous fish market in Spain, then no generalisation can be made for these species, especially when the contents were highly variable as, e.g., in the case of tuna, goatfish and thornback ray.

MeHg values obtained through this study are in good agreement with results published in other studies performed in Spain. For example, values for tuna, mackerel, swordfish, sole, hake, poor cod, perch, and canned tuna, mackerel and octopus, are very closed to those referred by Sahuquillo et al. (2007) in a study

Table 3
MeHg determination in fortified natural fish samples with different levels of fat.

	Without addition	1 μg g^{-1} Hg(II) added		2 μg g^{-1} MeHg added	
	C_m (μg g^{-1})	C_m (μg g^{-1})	Recovery (%)	C_m (μg g^{-1})	Recovery (%)
Sole	0.231 ± 0.005	0.236 ± 0.012	100.5 ± 1.1	2.26 ± 0.06	105.3 ± 0.4
Gilt-head bream	0.231 ± 0.007	0.231 ± 0.009	100 ± 1	1.80 ± 0.02	83 ± 2
Tuna	4.5 ± 0.2	4.5 ± 0.3	97 ± 26	6.3 ± 0.3	97 ± 2

C_m: Mean (± standard deviation) corresponding to three independent analysis performed following the general procedure.

Table 4
MeHg content in natural fish samples obtained from the Valencian market.

Fish	Sampling campaign		
	1	2	3
Cod	0.267 ± 0.008 (87%)	0.103 ± 0.002 (98%)	0.04 ± 0.02 (94%)
Four-spot megrim	0.0425 ± 0.0005 (124%)	0.238 ± 0.009 (78%)	0.408 ± 0.009 (58%)
Gilt-head bream	0.062 ± 0.002 (94%)	0.067 ± 0.003 (84%)	0.292 ± 0.003 (57%)
Goatfish	0.0875 ± 0.0014 (60%)	0.079 ± 0.003 (82%)	0.75 ± 0.04 (63%)
Hake	0.126 ± 0.003 (129%)	0.109 ± 0.003 (87%)	0.116 ± 0.003 (87%)
Halibut	0.092 ± 0.002 (80%)	0.0739 ± 0.0006 (87%)	0.0748 ± 0.0002 (98%)
Mackerel	0.047 ± 0.004 (72%)	0.026 ± 0.002 (33%)	0.0633 ± 0.0002 (108%)
Canned mackerel	0.0602 ± 0.0011 (83%)	nq	0.048 ± 0.003 (118%)
Monkfish	0.286 ± 0.004 (75%)	0.3599 ± 0.0009 (90%)	0.233 ± 0.005 (89%)
Nile perch	0.042 ± 0.003 (129%)	0.0868 ± 0.0007 (90%)	0.0350 ± 0.0012 (93%)
Canned octopus	0.0362 ± 0.0012 (76%)	0.0568 ± 0.0012 (97%)	0.0311 ± 0.0014 (118%)
Panga	nq	nq	nq
Poor cod	0.2151 ± 0.0004 (75%)	0.235 ± 0.003 (91%)	0.314 ± 0.006 (92%)
Sea bream	0.185 ± 0.002 (84%)	0.44 ± 0.04 (79%)	0.309 ± 0.004 (84%)
Sole	0.0797 ± 0.008 (89%)	0.0462 ± 0.0010 (95%)	0.085 ± 0.003 (88%)
Swordfish	0.30 ± 0.02 (96%)	1.24 ± 0.03 (82%)	0.76 ± 0.02 (67%)
Thornback ray	0.059 ± 0.008 (105%)	0.052 ± 0.003 (85%)	0.1299 ± 0.0005 (89%)
Tuna	0.27 ± 0.02 (88%)	1.058 ± 0.014 (83%)	0.67 ± 0.009 (67%)
Canned tuna	0.0499 ± 0.0009 (103%)	0.2479 ± 0.0008 (91%)	0.54 ± 0.03 (88%)

Concentration expressed in $\mu g\ g^{-1}$ referred to fresh sample as the mean (± standard deviation) of three independent analysis performed following the general procedure. Values on parenthesis are the percentage of MeHg respect to the total mercury content (García et al., 2013).

Table 5
Mean level and Estimated Weekly Intake for MeHg in Spain considering the different groups of fish samples.

Groups	Fish consume (g/person · day)[a]	MeHg median (mg/kg)[b]	EWI (μg/person)[c]	Weekly contribution to TWI (%)[d]
Fatty fish	15.68	0.57	62.38	69
Lean fish	41.41	0.10	29.98	33
Canned food	11.43	0.05	3.99	4

[a] Diary consume from the Spanish National Survey on Dietary Intake (Agencia Española de Seguridad Alimentaria y Nutrición (AESAN) 2013).
[b] Median MeHg content estimated from data showed in Table 4 distributed in three groups.
[c] Estimated weekly intake, EWI (μg/person) = fish consume (g/person · day) × 7 (days) × MeHg median (mg/kg).
[d] Weekly contribution to Tolerable Weekly Intake (TWI) calculated as EWI/TWI × 100. TWI is 91 μg/person for an adult of 70 kg body weight (European Food Safety Authority (EFSA), 2012).

with samples acquired at the Valencian market analysed by AAS; and the same concentration ranges were obtained for hake, mackerel, panga, perch, swordfish, tuna and canned octopus, tuna and mackerel by Olmedo et al. (2013) in a study with samples acquired in Granada and analysed by LC-ICP-MS.

Regarding the relative percentage of MeHg to the total Hg content in the analysed samples it ranged between 33 and 129 percent, being mackerel the lowest percentage obtained for MeHg. Most of the samples were between 69 and 105 percent. This is in a good agreement with EFSA guideline which reported that more than 90 percent of total mercury contained in fish is actually present as MeHg (European Food Safety Authority (EFSA), 2012). The variability of data can be explained due to the different species evaluated and their capture in different times of the year. Furthermore the variability within the same species can be attributable primarily to different feeding, size, origin and age of fish. Additionally data for MeHg percentage above 100 percent obtained in few cases evidenced the problems arising from the comparison of MeHg concentrations with total Hg ones and thus, the accumulation of errors can provide excess values.

There is not a specific legislation in the European Union for MeHg concentration in fish, but in our case, values found for tuna in campaign 2, swordfish in campaign 2 and goatfish in campaign 3 were higher than the maximum levels fixed for total mercury (European Comission (EC), 2008), thus evidencing the need to carefully control MeHg in fish.

3.8. Methylmercury intake in Spain

Population groups who frequently consume large predatory fish may have a considerably high intake of MeHg and exceed the Tolerable Weekly Intake (TWI). Since fish is the main Hg contributor in the diet of most people, there is an urgent need to regulate fish intake.

In order to perform a risk assessment of MeHg intake from fish consumption, it has been considered the median MeHg content in the studied species, distributed in three groups (fatty fish, lean fish and canned food), and the corresponding rate of fish consumption from the Spanish National Survey on Dietary Intake (Agencia Española de Seguridad Alimentaria y Nutrición (AESAN), 2011). Calculations were made for a person of 70 kg body weight (see Table 5).

The estimated weekly intakes (EWI) of MeHg were compared with the current TWI (91 μg/person for an adult of 70 kg bw). For each group individually, EWI was lower than TWI. However, considering the total contribution from all the groups, the TWI was overcome (96.4 μg/person).

So, it can be concluded that the consumption of fish could be considered safe although the TWI might be exceeded mainly by consumers of the predatory fish species. This supports the EFSA guidelines for particularly vulnerable population groups, such as pregnant women and infants, to limit the intake of these fish (European Food Safety Authority (EFSA), 2012).

4. Conclusions

Studies carried out evidenced the suitability of the modified extraction procedure to separate MeHg from fish samples by using HBr through a liquid–liquid extraction with toluene followed by a back extraction with L-cysteine and to measure by TDA-AAS.

The methodology was fully validated and applied to samples of the Spanish market, being obtained results for fatty, lean and canned fish. Additionally, their contribution to the MeHg estimated intake was evaluated considering the fish consumption data reported by the Spanish National Survey of Dietary Intake, showing the risk of overcome the TWI.

From an environmental point of view it can be concluded that the previously employed method has been improved with a drastic reduction of the use of reagents and then a green score of 73 points makes possible to classify it as acceptable green analysis.

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3. Determinación de arsénico, antimonio, bismuto, selenio y teluro

3.1. Determinación de arsénico, antimonio, bismuto, selenio y teluro en potitos

Evaluation of the content of antimony, arsenic, bismuth, selenium, tellurium and their inorganic forms in commercially baby foods

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Evaluation of the Content of Antimony, Arsenic, Bismuth, Selenium, Tellurium and Their Inorganic Forms in Commercially Baby Foods

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Abstract Baby foods, from the Spanish market and prepared from meat, fish, vegetables, cereals, legumes, and fruits, were analyzed to obtain the concentration of antimony (Sb), arsenic (As), bismuth (Bi), and tellurium (Te) as toxic elements and selenium (Se) as essential element. An analytical procedure was employed based on atomic fluorescence spectroscopy which allowed to obtain accurate data at low levels of concentration. Values of 14 commercial samples, expressed in nanograms per gram fresh weight, ranged for Sb 0.66–6.9, As 4.5–242, Te 1.35–2.94, Bi 2.18–4.79, and Se 5.4–109. Additionally, speciation studies were performed based on data from a non-chromatographic screening method. It was concluded that tellurium and bismuth were mainly present as inorganic forms and selenium as organic form, and antimony and arsenic species depend on the ingredients of each baby food. Risk assessment considerations were made by comparing dietary intake of the aforementioned elements through the consumption of one baby food portion a day and recommended or tolerable guideline values.

Keywords Infant feeding · Dietary intake assessment · Food security · Toxic elements and micronutrient

Introduction

New lifestyles in industrialized countries lead to new feeding habits. Parents have generally less time to prepare home-cooked foods than in the past encouraging the use of commercial baby foods, which play a basic role in the infant's diet [1]. Infant growth and the development of their nervous, reproductive, digestive, respiratory, and immune systems critically depend on nutritional imbalances; moreover, children are more susceptible to exposure of contaminants than adults due to their high intestinal absorption capability and low effective excretion [2].

Therefore, commercial baby foods should guarantee suitable proportions of essential elements and the absence of non-essential or toxic elements coming from raw materials and additives used in the product formulation, derived from manufacture or from poor-quality production processes.

Antimony (Sb) is hazardous to human health, and the trioxide form is classified as possibly carcinogenic by the International Agency for Research on Cancer [3]. Sb can be easily taken up by plant roots; hence, rice and vegetables, especially leafy vegetables, are the major source of exposure to Sb for humans [4].

Arsenic (As) is ranked by the Agency for Toxic Substances and Disease Registry as rank 1 on the Priority List of Hazardous Substances [5], and it is classified by IARC as a human carcinogen [6]. For the general population, drinking water is the major source of exposure to As followed by fish consumption, in which high levels occur as non-toxic arsenobetaine, as well as cereals in which As occurs as toxic inorganic forms. Presence of arsenic in animals or plants comes from contaminated water and soils, from some additives in animal feed, and from the use of pesticides, such as arsanilic acid, allowed in countries like USA and China [7]. Currently, in the EU, there are maximum levels established by law only for arsenic in rice and rice products from 0.1 to 0.3 mg kg^{-1} [8]; the Joint FAO/WHO Expert Committee on Food Additives withdrew in 2010 the PTWI of 15 μg kg^{-1} body weight for inorganic arsenic because they considered it too risky [9].

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Studies evidencing tellurium (Te) toxicity have recently appeared since this element is used in increasing amounts in industry and agriculture. Te determination in food is particularly important because some vegetables, such as onions and garlic, accumulate it [10].

Bismuth (Bi) is not considered as an essential element by the Food and Agricultural Organization-World Health Organization (FAO/WHO), it has an unknown physiological function . Although Bi exposure is basically related to the use of medicine and cosmetic products, some authors warn of its presence in vegetables, milk, and dairy products [11].

Selenium (Se) is an essential element at low concentrations. It protects against oxidative damage, inhibits the toxicity of some heavy metals, and regulates the thyroid hormone action and the redox status of vitamin C and other molecules. Meat, fish, and vegetables, with elevated protein content, are the most important Se sources for humans [12].

The development of analytical methods for the determination of traces of the aforementioned elements is a challenge because of the extremely low concentrations at which they are present in natural and processed foods and the strong interferences from sample matrices. The common characteristic between Sb, As, Bi, Se, Sb, and Te is that they generate volatile covalent hydrides, which allows the selective separation of these elements from matrices and their complete transfer to the detector. So, hydride generation (HG) coupled to non-dispersive atomic fluorescence spectroscopy (AFS) makes possible to get an excellent sensitivity in their analysis [13]. In addition, dry ashing completely destroys organic matter avoiding interferences in hydride generation and fluorescence measurement. Therefore, the use of high dilution factors is unnecessary and the analysis at ultra-trace levels is possible.

It is known that the toxic effects and bio-availability of the mineral elements depend on their different chemical forms present in the samples [14]. So, it is necessary to develop sensitive and reliable methods to identify and quantify the different chemical species. For example, Sb(III) is approximately 10 times more toxic than Sb(V), inorganic arsenic species are about 100 times more toxic than the organic ones, and inorganic selenium forms are more toxic than organic ones, with Se(IV) being considered more toxic than Se(VI).

Table 1 summarizes previously published data on total content of As, Se, and Sb and inorganic As in baby foods. However, in our knowledge, there is no available information about Te and Bi content in this kind of samples and few works showed contents of inorganic As. So, the main objective of this work was the development and application of a non-chromatographic procedure for the speciation of inorganic forms of Sb, As, Bi, Se, and Te in commercially prepared baby food and the estimation of their contribution to the dietary intake of infants.

Material and Methods

Apparatus, Reagents, and Samples

A continuous-flow hydride-generation atomic-fluorescence spectrometer model PSA Millennium Excalibur 10.055 from PS Analytical (Kent, UK) was used for the analytical determinations. Boosted discharge hollow-cathode lamps of As, Sb, Se, Te, and Bi, all from Photron (Victoria, Australia), were employed as excitation sources. Other equipment included a freeze-drier Telstar Cryodos system (Barcelona, Spain), a Selecta sand bath (Barcelona, Spain), and a Lenton ECF 12145 muffle furnace equipped with a Eurotherm 2416 controller Biometa (Oviedo, Spain). A Bransonic ultrasound water bath (Danbury, CT, USA), operated at 130 W and 50–60 Hz, was used for sample extraction and a Mettler Toledo analytical balance (Barcelona, Spain) to weight samples and standards.

All reagents used were of reagent grade, and all solutions were prepared in ultrapure water with a minimum resistivity of 18.2 MΩ cm obtained from a Milli-Q Millipore system (Bedford, USA). The 1000 mg L^{-1} Sb(III) stock solution was prepared from $C_4H_4KO_7Sb{\cdot}0.5H_2O$ and Sb(V) stock standard from H_6KO_6Sb, both from Fluka (Buchs, Switzerland). The 1000 mg L^{-1} As(III) stock solution was prepared from As_2O_3 from Riedel de Häen (Hannover, Germany). The 1000 mg L^{-1} As(V), Se(IV), Te(IV), and Bi(III) standard solutions were supplied by Merck (Darmstadt, Germany). Se(VI) and Te(VI) standards were prepared by dissolving Na_2SeO_4 and H_6O_6Te, both from Fluka. For sample preparation, HCl 37% from Merck and HNO_3 65% from Scharlau (Barcelona, Spain) were used. The ashing agent employed was a suspension of $Mg(NO_3){\cdot}6H_2O$ and MgO (extra pure), both from Scharlau. Solutions of KI from Merck and ascorbic acid and KBr from Scharlau were used in sample preparation. Sodium tetrahydroborate from Fluka, dissolved in NaOH from Scharlau, was used as reductant to generate the corresponding hydrides. For extraction of the inorganic species, a 1 mol L^{-1} solution of H_2SO_4 from Scharlau and a 0.1% (w/v) solution of the disodium salt of ethylenediaminotetraacetic acid from Panreac (Barcelona, Spain) were used. Argon C-45 supplied by Carburos Metálicos (Barcelona, Spain) with purity higher than 99.995% was employed as carrier and drier gas.

Fourteen commercial baby foods, distributed under two different brand names, were acquired in local markets of Burjassot (Spain). Purees were made with different kinds of foods: fruit, meat, fish, cereals, legumes, and vegetables. Specific ingredients and nutritional information concerning each single sample assayed are provided in Table 2. Baby foods were freeze-dried for approximately 48 h at a chamber pressure of 0.05 mbar, pulverized, and homogenized using a conventional food mill. Dry samples were stored in polyethylene bottles and kept in a desiccator until their analysis.

Table 1 Literature precedents on the analysis of baby foods for the determination of total As, Se and Sb and inorganic As

Reference	Country	Food components	t-As (ng g^{-1})	i-As (ng g^{-1})	t-Se (ng g^{-1})	t-Sb (ng g^{-1})	Technique
[15]	UK	Fish, vegetables, rice	<10		80–290	<6	ICP-MS
		Meat, vegetables, rice	<10		<22–290		
[16]	Spain	Fish, vegetables, rice	225, 479				HG-AAS
		Meat, vegetables, rice	49, 55				
[17]	Spain	Fish, vegetables	105–280	nd			HG-AFS
		Meat, vegetables	2–85				
[18]	Spain	Fish, vegetables, rice	105–280	nd			ET-AAS
[19]	Spain, China, USA, UK	Fish	198–220		50–181		ICP-MS
		Meat	9–26		19–144		
		Rice	70–506		<13–225		
[20]	Spain, China, USA, UK	Fish	159–2310	nd-11 7–11			ICP-MS
		Meat	9–26				
		Rice	70–394				
[21]	Spain	Fish, vegetables	0.2–2.4				ET-AAS
[22]	USA	Meat, rice	2.3, 18.5				ICP-MS
		Fruits	16.6				
		Vegetables	3.7				
		Rice	11.9				
[23]	Argentina	Rice, cereals, milk			34–67		PC-INAA-CSS
[24]	Norway	Fruit, fish	<1.10				HR-ICP-MS
[25]	Spain	Fish, vegetables, rice			209–258[a]		HG-AAS
		Meat, vegetables, rice			54–153[a]		
		Vegetables			20, 82[a]		
[26]	USA	Rice, cereals, fruits, and vegetables	<1–24				ICP-MS
[27]	Spain	Fish			21.5–72.8		ET-AAS

[a] Expressed as nanograms per gram referred to dry weight

ET-AAS electrothermal atomic absorption spectrometry, *HG-AAS* hydride generation atomic absorption spectrometry, *HG-AFS* hydride generation atomic fluorescence spectrometry, *ICP-MS* inductively coupled plasma mass spectrometry, *PC-INAA-CSS* pseudo-cyclic instrumental neutron activation analysis method with Compton suppression spectrometry, *t-As* total arsenic, *i-As* inorganic arsenic, *nd* not detected

Certified reference material TORT-2 (lobster hepatopancreas) was obtained from the National Research Council of Canada (NRC) and used to check the accuracy of the method.

Experimental Procedures

Determination of Total Sb, As, Bi, Se, Sb, and Te

According to a previously described procedure by Cervera et al. [28], approximately 1 g of sample was accurately weighed and treated with 2.5 mL of ashing aid suspension containing 20% (*w*/*v*) $Mg(NO_3)_2{\cdot}6H_2O$ plus 2% (*w*/*v*) MgO, and 5 mL 50% (*v*/*v*) HNO_3. The mixture was evaporated to dryness in a sand bath and mineralized in a muffle furnace at 450 °C with a gradual increase in temperature. The white ashes were wet with 1 mL water and dissolved in 10 mL 10% (*v*/*v*) HCl. For As and Sb determination, an aliquot of the sample was prepared in a medium containing 3.5 mol L^{-1} HCl, 1% (*w*/*v*) KI, and 0.2% (*w*/*v*) ascorbic acid for species reduction. For the Se, Te, and Bi determinations, an aliquot sample was fitted to 4 mol L^{-1} HCl and 1% (*w*/*v*) KBr as reductant.

Conditions for hydride generation atomic fluorescence spectroscopy instrument were adjusted according to previous studies focused to improve the analytical signals and their sensitivity [29, 30].

Arsenic, Se, Sb, Te, and Bi concentrations were determined using external calibration. Employed standard solutions were prepared daily by sequential dilution of stock ones and treated in the same way than diluted samples. Quality assurance of data was assured by measuring the samples in triplicate. Additionally, a certified reference material and blank solutions were analyzed together with every set of samples.

Determination of Inorganic Chemical Forms

Approximately 1 g freeze-dried sample was accurately weighed inside a 50-mL polyethylene tube, and 10 mL 1 mol L^{-1} H_2SO_4 was added to the tube. The mixture was sonicated for 10 min, and the sulfuric extract was separated by centrifugation at 3500 rpm for 10 min. The solid residue was washed with 10 mL 0.1% (*w*/*v*) EDTA; this suspension was centrifuged for an additional 10 min, and the supernatant was mixed with the previous extract. Four aliquots were taken from the final extract of samples. For As(III), As(V), Sb(III),

Table 2 Description of baby food samples analyzed through this study

Product name	Ingredients	Nutritional information/100 g
Fruit based		
Peach and banana	Puree of fruits (100 g/100g), (peach, banana, and grape juice from concentrate), corn starch, and vitamin C.	Energy 326 kJ/76 kcal, protein 0.8 g, carbohydrate 17.5 g, sugar 15 g, fat 0.1 g, saturates 0.0 g, sodium 11 mg, fiber 1.4 g, and vitamin C 25 mg.
Meat based		
Turkey with vegetables	Water, turkey (15%), potatoes, peas, tomato, rice, onion, corn starch, olive oil (0.9%), vegetable oil, and salt.	Energy 300 kJ/71 kcal, protein 3.9 g, carbohydrate 8.3 g, sugar 1.0 g, fat 2.5 g, saturates 0.6 g, sodium 118 mg, and fiber 0.8 g.
Stew of lamb	Water, potatoes, carrot, lamb (10%), peas, onion, corn starch, olive oil (0.7%), vegetables oil, salt, parsley, and garlic.	Energy 263 kJ/62 kcal, protein 2.8 g, carbohydrate 7.7 g, sugar 1.6 g, fat 2.3 g, saturates 0.8 g, sodium 108 mg, and fiber 1.2 g.
Puree of vegetables with chicken and beef	Potato, water, carrots, leek, peas, chicken (6%), onion, beef (3%), olive oil (2%), corn starch, and salt.	Energy 299 kJ/71 kcal, protein 3.2 g, carbohydrate 8.1 g, sugar 2.3 g, fat 2.9 g, saturates 0.6 g, sodium 57 mg, and fiber 1.5 g.
Carrots with rice in poultry broth	Broth of poultry (45%), carrots (34%), chicken (10%), rice (5%), onion, corn starch, olive oil (0.5%), vegetables oil, parsley, salt, and laurel.	Energy 248 kJ/59 kcal, protein 3.0 g, carbohydrate 7.9 g, sugar 3.3 g, fat 1.7 g, saturates 0.3 g, sodium 97 mg, and fiber 1.2 g.
Rice and chicken	Water, powdered milk, chicken (12%), potato, carrot, rice (4%), corn starch, olive oil (1.5%), vegetables oil, lactose, salt, and soya.	Energy 320 kJ/76 kcal, protein 3.2 g, carbohydrate 8.7 g, sugar 2.1 g, fat 3.2 g, saturates 0.8 g, sodium 73 mg, and fiber 0.4 g.
Chicken and rice	Water, chicken (15%), rice (6%), carrot, corn starch, tomato, onion, olive oil (0.8%), vegetables oil, celery, and salt.	Energy 280 kJ/67 kcal, protein 3.6 g, carbohydrate 7.0 g, sugar 0.6 g, fat 2.7 g, saturates 0.7 g, sodium 115 mg, and fiber 0.5 g.
Fish based		
Sole with béchamel	Water, potatoes, skimmed milk, sole (12%) tomato, butter, corn starch, onion, celery, olive oil (0.4%), vegetable oil, and salt.	Energy 300 kJ/71 kcal, protein 3.5 g, carbohydrate 8.3 g, sugar 1.2 g, fat 2.7 g, saturates 1.1 g, sodium 111 mg, and fiber 0.5 g.
Selected vegetables and sea bass	Vegetables (58%) (carrot, potato, peas, and onion), water, sea bass (12%), powdered milk, olive oil (2.5%), corn starch, vegetables oil, lactose, salt, and soya.	Energy 292 kJ/70 kcal, protein 3.0 g, carbohydrate 8.1 g, sugar 3.1 g, fat 2.8 g, saturate 0.5 g, sodium 70 mg and fiber 1.6 g.
Selected vegetables and monkfish	Vegetables (60.8%) (carrot, potato, peas, onion, green bean, and leek), water, monkfish (12%), powdered milk, olive oil (2%), corn starch, vegetables oil, lactose, salt, and soya.	Energy 290 kJ/70 kcal, protein 2.9 g, carbohydrate 8.3 g, sugar 2.4 g, fat 2.8 g, saturates 0.4 g, sodium 71 mg, and fiber 1.3 g.
Cream of vegetables with monkfish	Water, potatoes, skimmed milk, monkfish (10%), tomato, onion, butter, corn starch, celery, olive oil (0.4%), vegetable oil, and salt.	Energy 299 kJ/71 kcal, protein 2.8 g, carbohydrate 8.7 g, sugar 1.2 g, fat 2.7 g, saturates 1.3 g, sodium 110 mg, and fiber 0.5 g.
Rice with hake	Water, potatoes, hake (12%), rice (7.0%), onion, olive oil (1.2%), vegetables oil, celery, and salt.	Energy 300 kJ/71 kcal, protein 2.9 g, carbohydrate 9.1 g, sugar 0.4 g, fat 2.6 g, saturates 0.5 g, sodium 110 mg, and fiber 0.5 g.
Vegetable based		
Cream of green beans with potatoes	Green beans (40%), skimmed milk (32%), potatoes (27%), onion, cream of milk (3%), rice starch, olive oil (0.25%), vegetable oil, and salt.	Energy 281 kJ/66 kcal, protein 2.4 g, carbohydrate 10.7 g, sugar 3.4 g, fat 1.6 g, saturates 0.5 g, sodium 115 mg, and fiber 1.4 g.
Cream of vegetables with pasta	Skimmed milk (32%), water, beans (14%), peas (10%), onion (5%), pasta (4%), rice, cream (3%), corn starch, olive oil (0.5%), vegetable oil, and salt.	Energy 320 kJ/76 kcal, protein 2.5 g, carbohydrate 10.9 g, sugar 3.4 g, fat 2.2 g, saturates 0.8 g, sodium 100 mg, and fiber 1.4 g.

and Sb(V) determination, the medium was adjusted to 3.5 mol L^{-1} HCl; one aliquot was prepared in a medium containing 1% KI and 0.2% ascorbic acid and was let to react for 30 min; and another one was analyzed by HG-AFS without pre-reduction. In the case of Se(IV), Se (VI), Te(IV), and Te(VI), one aliquot was prepared in a medium containing 4 mol L^{-1} HCl plus 1% (*w*/*v*) KBr; this solution was warmed for 30 min at 80 °C before instrumental determination, and

another one was analyzed directly in a medium containing 4 mol L^{-1} HCl.

Results and Discussion

Characteristics of the Analytical Method

The employed methodology for the analysis of baby food purees by HG-AFS has been previously used successfully in the analysis of several food matrices. Matos-Reyes et al. analyzed vegetables, cereals, pulses, and garlic [31–33], Sousa Ferreira et al. analyzed mushrooms and garlic [34, 35], Ródenas-Torralba et al. analyzed milk [36], and Gómez et al. analyzed marine organisms [37]. Additionally, the accuracy of the methodology was evaluated from the analysis of a certified reference material, TORT-2 lobster hepatopancreas. Found values (μg g^{-1}) were As 20.3 ± 1.4 and Se 5.65 ± 0.93, which are in good agreement with certified values (μg g^{-1}) of As 21.6 ± 1.8 and Se 5.63 ± 0.67.

The method detection limit (LOD) and quantification limit (LOQ) were calculated as three and ten times, respectively, the standard deviation of the fluorescence signal of ten blank solutions divided by the slope of the calibration line measured in the experimental conditions for each studied element. The LOD and LOQ values were determined for dried samples, taking into consideration the amount of sample and dilution factor involved in the methodology. The precision of the method was evaluated from the relative standard deviation (RSD) calculated for three independent analyses of real samples employed through this study. Limits of detection found were in the range between 0.9 and 2.3 ng g^{-1} (dry weight) for total As, Sb, Se, Te, and Bi determination and in the range between 0.7 and 1.9 ng g^{-1} (dry weight) for inorganic species determination. RSD values for total As, Sb, Se, Te, and Bi determination were between 0.2 and 15%, with the exception of Te and RSD values for inorganic species determination which were between 0.5 and 28% with the exception of Sb(V) (see Table 3). So, the analytical procedures employed provided analytical characteristics good enough for the quantification of the nine species considered in the analyzed samples at few nanogram per gram levels, with a suitable precision.

Analyses of Commercial Baby Foods

Table 4 shows the results obtained for the considered elements in 14 commercially available baby foods as the average and corresponding standard deviation of three independent analyses, expressed in nanograms per gram fresh weight taking into account the moisture content in each case which was around 84%. These data can be compared with those previously published by other authors summarized in Table 1.

Table 3 Analytical figures of merit obtained for As, Se, Sb, Te, and Bi and inorganic species determination by HG-AFS after dry ashing of samples

Element	LOD (ng g^{-1})	LOQ (ng g^{-1})	RSD (%)
As	0.9	3.0	0.2–8
Sb	0.9	3.0	0.4–15
Se	1.8	6.0	0.5–7
Te	1.9	6.3	1.2–28
Bi	2.3	7.7	0.5–10
As(III)	0.9	3.0	1.5–12
As(V)	0.7	2.3	0.6–22
Sb(III)	0.9	3.0	1.0–28
Sb(V)	1.0	3.3	2.8–43
Se(IV)	1.8	6.0	0.5–16
Se(VI)	1.2	4.0	–
Te(IV)	1.9	6.3	1.8–15
Te(VI)	0.8	2.7	–

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as 3 or 10 times, respectively, standard deviation of ten consecutives blanks (ng mL^{-1}) and per dilution and mass factor conversion (ng g^{-1}). The relative standard deviation (RSD) was established as the range of the variation coefficient found for three replicate analyses of commercially available samples

Antimony

The Sb concentration ranged from 0.6 to 6.9 ng g^{-1} referring to fresh weight (fw). In general, data are similar for all samples. It could be noticed that Sb content in meat-based foods, with an average of 1.5 ng g^{-1}, was higher than in fish-based products with an average of 0.8 ng g^{-1}. The highest content of Sb was 6.9 ng g^{-1} in the cream of green beans and potatoes, followed by 4.1 ng g^{-1} in peach and banana puree.

Arsenic

The total As concentration ranged from 4.5 to 242 ng g^{-1} (fw). As expected, fish-based purees presented the highest values, with contents in the range 164.9–242 ng g^{-1}. In this kind of baby foods, fish is clearly the main contribution to As content. In fact, taking into account 10–12% of fish in baby food formulation, results are consistent with the general reported values of As in fish, e.g., 2.37 and 16.5 μg g^{-1} in hake, 5.54 μg g^{-1} in sole, or 11.1 μg g^{-1} in monkfish [38, 39]. Some variability in results could be attributed to natural factors which are critical in mineral content in fish, such as size or geographical origin.

Purees based on meat, vegetables, or fruits presented arsenic contents in a very narrow range, 4.5–18 ng g^{-1}. Taking into account that the amount of meat in baby food formulation is 9–15% of the total weight, these values could be consistent with low values of As reported in meat, e.g., 116 ng g^{-1} [39].

Table 4 Total Sb, As, Se, Te, and Bi contents (ng g^{-1}) in baby food samples

Sample	Sb	As	Se	Te	Bi
Peach and banana	4.10 ± 0.14	14.04 ± 0.03	5.4 ± 0.4	2.0 ± 0.1	3.4 ± 0.3
Turkey with vegetables	2.3 ± 0.3	14.1 ± 1.1	22.5 ± 1.1	1.9 ± 0.3	2.6 ± 0.2
Stew of lamb	1.646 ± 0.007	4.5 ± 0.3	15.16 ± 0.10	1.9 ± 0.2	2.57 ± 0.09
Puree of vegetables with chicken and beef	1.48 ± 0.15	6.21 ± 0.15	31.3 ± 1.5	2.4 ± 0.7	3.4 ± 0.3
Carrots with rice in poultry broth	1.16 ± 0.06	18.0 ± 1.0	24.6 ± 0.4	1.5 ± 0.2	2.42 ± 0.12
Rice and chicken	1.29 ± 0.10	13.32 ± 0.05	21.1 ± 0.5	1.7 ± 0.2	3.298 ± 0.015
Chicken and rice	0.72 ± 0.08	16.84 ± 0.05	36.3 ± 0.5	1.49 ± 0.08	2.2 ± 0.2
Sole with béchamel	0.66 ± 0.05	209.9 ± 1.4	43.9 ± 0.7	1.35 ± 0.09	2.42 ± 0.15
Selected vegetables and sea bass	1.06 ± 0.12	208 ± 2	49.4 ± 1.8	1.69 ± 0.13	2.18 ± 0.05
Selected vegetables and monkfish	0.69 ± 0.10	164.9 ± 1.3	64.5 ± 0.3	1.8 ± 0.5	3.0 ± 0.3
Cream of vegetables with monkfish	1.30 ± 0.05	242 ± 5	109 ± 3	2.58 ± 0.11	4.79 ± 0.05
Rice with hake	0.85 ± 0.08	215 ± 6	85.7 ± 1.1	2.0 ± 0.3	3.05 ± 0.05
Cream of green beans with potatoes	6.9 ± 0.5	13.84 ± 0.08	8.83 ± 0.11	2.94 ± 0.08	3.50 ± 0.02
Cream of vegetables with pasta	1.3 ± 0.2	11.5 ± 0.2	28.0 ± 0.2	2.45 ± 0.03	3.1 ± 0.9

Results are the average ± standard deviation of three replicates. Results are referred to fresh weight

Arsenic contribution in vegetable and fruit purees was low which shows that any polluted water or soils were involved in the cultivation process. Additionally, it is known that As often remains in the peel of vegetables [40].

In the last years, the presence of As in rice and other cereals has been a matter of concern; thus, an important number of papers have appeared in the literature. Arsenic varies from tens to hundreds of nanograms per gram as a function of the rice provenance [41, 42]. From fish purees containing rice, no deductions can be done because of the important contribution of fish to total As. However, in meat-based purees, those with rice (average 16.4 ng g^{-1}) can be highlighted with respect to those without rice (average 8.6 ng g^{-1}).

Bismuth

The Bi concentration found through this study ranged from 2.18 to 4.79 ng g^{-1} (fw), all data being similar for all types of purees.

Selenium

The Se concentration ranged from 5.4 to 109 ng g^{-1} (fw) in the studied samples. Fish-based purees presented the highest values in the 44–109 ng g^{-1} range, followed by meat-based purees in the 15–36 ng g^{-1} range. Purees based on vegetable and fruit presented the lowest (below 10 ng g^{-1}) contents with the exception of puree with pasta. In the present case, Se content in all types of ingredients is similar; thus, all of them contributed significantly. For example, Se concentrations around 200 ng g^{-1} for fish, 76–190 ng g^{-1} for meat [43], 15–270 ng g^{-1} for legumes [31], 199–826 ng g^{-1} for pasta [43], and 28–236 ng g^{-1} for rice [44] have been reported.

Tellurium

The Te concentration ranged from 1.35 to 2.94 ng g^{-1} fw, and data were very similar for all types of purees. In our knowledge, there are no data available in the literature for Te content in infant foods and, in general, few data have been provided for foodstuffs. The amount of Te in purees formulated as cream with a high content of skimmed milk could be highlighted. Te contents in these purees, vegetables with monkfish, green beans with potatoes, and vegetables with pasta were 2.58, 2.94, and 2.45 ng g^{-1}, respectively, which are in good agreement with reported data for Te in milk [30].

Speciation

Six baby foods were selected for speciation. Table 5 shows the levels of Sb(III), Sb(V), As(III), As(V), Bi(III), Se(IV), Se(VI), Te(IV), and Te(VI) in samples expressed in nanograms per gram fresh weight. It can be seen that in most samples the predominant species was As(III), in the case of fish being of the same order than As(V). The sum of the contents of Sb(III) and Sb(V) agree well with the total content determined by previous dry mineralization in some cases (hake and monkfish samples), while in others it provides an important part of total Sb but not the whole content. Therefore, we can conclude that Sb organic species were also present in these baby foods. The predominant specie was the highest oxidation state, unlike the case of As.

With respect to Se and Te, the predominant inorganic species were those with the lowest oxidation state, but for Se the organic forms represent more than 90%. On the contrary, Te inorganic forms were higher than organic ones.

Table 5 Inorganic species content (ng g^{-1}) in baby food sample

Sample	[Sb(III)]	[Sb(V)]	[As(III)]	[As(V)]	[Se(IV)]	[Se(VI)]	[Te(IV)]	[Te(VI)]	[Bi(III)]
Rice with hake	<LOD	1.25 ± 0.18	13.6 ± 0.3	12.96 ± 0.08	<LOD	<LOD	<LOD	<LOD	3.15 ± 0.13
Puree of vegetables with chicken and beef	<LOD	0.7 ± 0.3	1.04 ± 0.13	0.58 ± 0.13	0.480 ± 0.048	<LOD	1.44 ± 0.11	<LOD	2.64 ± 0.10
Cream of green beans with potatoes	0.829 ± 0.008	1.15 ± 0.03	10.45 ± 0.16	3.06 ± 0.13	0.644 ± 0.003	<LOD	2.1 ± 0.3	<LOD	3.2 ± 0.2
Cream of vegetables with pasta	0.29 ± 0.03	0.17 ± 0.02	5.4 ± 0.5	3.4 ± 0.5	0.82 ± 0.13	<LOD	2.7 ± 0.3	<LOD	3.2 ± 0.2
Cream of vegetables with monkfish	0.8 ± 0.2	0.42 ± 0.10	0.715 ± 0.048	0.73 ± 0.03	0.66 ± 0.06	<LOD	1.8 ± 0.2	<LOD	2.88 ± 0.08
Rice and chicken	0.43 ± 0.06	0.50 ± 0.08	2.22 ± 0.14	1.63 ± 0.19	0.37 ± 0.03	<LOD	1.82 ± 0. 03	<LOD	2.83 ± 0.18

Results are the average ± standard deviation of three replicates. Results are referred to fresh weight

Table 6 Total content (ng g^{-1}) and percentage of inorganic species in baby food samples

Sample	[Sb]	% Sb-i	[As]	% As-i	[Se]	% Se-i	[Te]	% Te-i	[Bi]	% Bi-i
Rice with hake	0.85 ± 0.08	133 ± 20	215 ± 6	12.2 ± 0.2	85.7 ± 1.1	nd	2.0 ± 0.3	nd	3.05 ± 0.05	102 ± 4
Puree of vegetables with chicken and beef	1.48 ± 0.15	46 ± 3	6.21 ± 0.15	27 ± 4	31.3 ± 1.5	1.58 ± 0.14	2.4 ± 0.7	60 ± 5	3.4 ± 0.3	79 ± 3
Cream of green beans with potatoes	6.9 ± 0.5	27.5 ± 0.4	13.84 ± 0.08	93.8 ± 0.2	8.83 ± 0.11	7.01 ± 0.03	2.94 ± 0.08	70 ± 11	3.50 ± 0.02	89 ± 6
Cream of vegetables with pasta	1.3 ± 0.2	40 ± 4	11.5 ± 0.2	88 ± 10	28.0 ± 0.2	3.3 ± 0.5	2.45 ± 0.03	126 ± 15	3.1 ± 0.9	116 ± 9
Cream of vegetables with monkfish	1.30 ± 0.05	93 ± 20	242 ± 5	0.572 ± 0.007	109 ± 3	0.57 ± 0.05	2.58 ± 0.11	67 ± 8	4.79 ± 0.05	57.4 ± 1.6
Rice and chicken	1.29 ± 0.10	74 ± 11	13.32 ± 0.05	30 ± 4	21.1 ± 0.5	1.82 ± 0.06	1.7 ± 0.2	111 ± 2	3.298 ± 0.015	89 ± 6

Results are the average ± standard deviation of three replicates. Results are referred to fresh weight

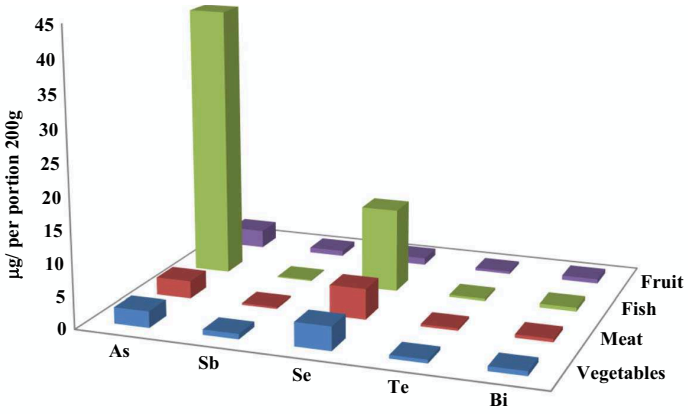

Fig. 1 Estimated intake (EI) of As, Bi, Se, Sb, and Te provided by the consumption of one portion of baby food (200 g) per day

	Sb	As	Bi	Se	Te
Vegetables	0.82	2.53	0.73	3.68	0.54
Meat	0.28	2.74	0.52	4.71	0.36
Fish	0.17	41.98	0.60	12.90	0.36
Fruit	0.82	2.81	0.68	1.08	0.40

The contents of Bi obtained by non-chromatographic speciation agree with the results of the determination of total Bi with the exception of cream of vegetables with monkfish puree. So, it can be concluded that there are no organic species of Bi in these samples of baby food.

Table 6 shows the total content of the five considered elements, expressed in nanograms per gram fresh weight, and the percentage of the inorganic species found. The contents of inorganic As are not very high, although in some cases worrying. Regarding the levels in fish, it may be concluded that, according to previous studies, most of the As present in the fish is in the form of arsenobetaine, a non-toxic chemical specie for humans which does not generate hydride; therefore, it is not detectable by HG-AFS. The percentage of inorganic As in the case of vegetable soup with monkfish sample was only 0.57%. However, for hake and rice samples, inorganic As increases to 12.1%, which shows the contribution of rice to inorganic As, for the similar total As content in both samples. The highest percentages of inorganic As were found in cream of green beans with potato puree and cream of vegetables with pasta puree, which were made with only vegetables and pasta. It justifies the interest in speciation of these foods, on the basis of diet at every stage of life, and in spite of the low levels of total As because the most toxic species represent from 88 to 93.8%. The contribution of inorganic As by rice was also evidenced on comparing the chicken, beef, and vegetable samples with rice and chicken samples, where the percentage varies from 27 to 30%.

It is noteworthy that in foods containing fish (hake and monkfish samples), 100% Sb is present as Sb-inorganic, and in the case of meat that level drops to 46 to 74%, taking into account that these purees are not the baby foods with highest levels of Sb and that they do not involve any risk to human health. On the other hand, low levels of inorganic Sb were

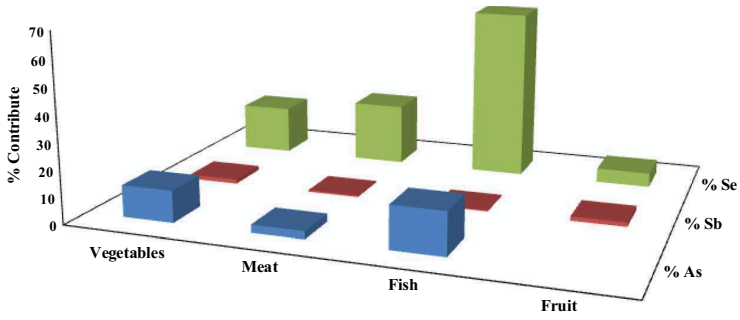

Fig. 2 Percentage of contribution to the tolerable and recommended infant daily intake (TDI and RDI) by the consumption of one portion of baby food. % TDI = EI / TDI * 100. TDI calculated for 8.4 kg body weight (bw) from i-As 15 µg kg^{-1} bw per week and Sb 6 µg kg^{-1} bw per day (Ródenas-Torralba et al. 2004). % RDI = EI / RDI * 100. RDI (7–12 months) = 20 µg day^{-1} (NIH 2014)

	Vegetables	Meat	Fish	Fruit
% As	12	3	16	-
% Sb	2	1	0	2
% Se	18	24	65	5

found in samples containing only vegetables (40 and 46%), which suggests the biomethylation of inorganic antimony by plants probably as a detoxification process. Since there are no limits to the contents of antimony and its species, assessing the risk to children is not possible.

Based on the percentages of Se-inorganic found in baby food, it can be concluded that most of the Se in these foods is present in the form of organic species as selenomethionine, selenocysteine, selenoamino acids, and selenoproteins. Reviewing in detail the results, it must be noticed that the percentages of the inorganic with respect to the total content in samples with fish were from nd to 0.57%, in samples with meat from 1.58 to 1.82%, and from 3.3 to 7% in samples containing only vegetables.

The main Te species were inorganic forms, with percentages higher than 60%. These results demonstrated the presence of organic tellurium species, which may be methylated species or Te amino acids; so far, it was reported in laboratory studies with yeast, bacteria, and fungi grown in tellurite-rich media.

Dietary Intake of Infants Consuming Commercial Baby Foods

The American Academy of Pediatrics and the World Health Organization recommend that complementary feeding could start at 6 months after a period of exclusive breastfeeding [45]. Commercial baby foods employed in this study are recommended for babies between 6 and 12 months old.

Figure 1 shows the estimated intake (EI) of Sb, As, Bi, Se, and Te in micrograms provided by the consumption of one portion of the studied baby food, distributed in four groups according to the main ingredient used in their elaboration. Calculations were done from median values of data shown in Table 4 and considering 200 g portion of baby food.

Additionally, as it can be seen in Fig. 2, the percentage of contribution of Sb, As, and Se to the daily intake as a function of the type of food was calculated. For Sb, it was evaluated through the comparison of the EI with the tolerable intake (TI) of 6 μg kg^{-1} bw a day, established by the FAO/WHO Expert Committee on Food Additives and considering 8.4 kg body weight for infants between 6 and 12 months [46, 47]. For As, it was evaluated considering the inorganic mean content in samples (Table 5) and the tolerable intake (TI) of inorganic As of 15 μg kg^{-1} body weight a week, in spite of this having been recently removed. Arsenic intake from fish-based baby food could be problematic only in the case that total As were as inorganic forms, but fortunately it represents only 16%. Low amounts of i-As in purees with meat represent around 3%. The highest percentage of i-As was present in purees with vegetables (88%), but total As content was very low and these samples contribute 12% which was not of concern if a regular consumption of baby foods was performed. The amount of Sb in all considered products was not significant.

Selenium was evaluated through the comparison of the EI with the recommended daily intake (RDI) of Se for infants aged of 7–12 months, 20 μg day^{-1}, suggested by the National Institutes of Health [12]. As it can be seen in Fig. 2, the contribution from fruit purees was low, and was medium from meat and vegetables purees, and fish purees provided a high content of Se.

There was not a reference value to evaluate the intake of Te and Bi in baby food samples.

Conclusion

Studies carried out evidenced the low content of toxic species of As, Sb, Se, Te, and Bi in commercial baby foods obtained from the Spanish market. From data of total concentrations of As, Sb, Se, Te, and Bi, values of As in baby foods containing fish should be highlighted. However, speciation studies showed that As in these samples, fortunately, was present mostly as organic species. Inorganic arsenic was present basically in vegetable samples. Bismuth and tellurium were present in inorganic forms, selenium as organic forms, and antimony in both organic and inorganic species.

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3.2. Determinación de bismuto y teluro en yogur

Comparison of sample treatment methods for bismuth and tellurium determination in yogurt

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Comparison of Sample Treatment Methods for Bismuth and Tellurium Determination in Yogurt

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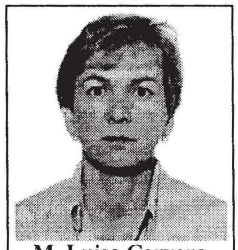

M. Luisa Cervera

Abstract:

Background: Dairy products are rich in nutrients that are essential for a high bone mineral density. Furthermore, several studies relate additional health benefits to the regular consumption of fermented milk products such as yogurt. However, Te and Bi have been determined in milk samples but there is not available information about Te and Bi determination in yogurt samples.

Methods: Three sample treatment approaches including: i) microwave-assisted digestion, ii) ultrasound-assisted extraction at room temperature, and iii) microwave assisted extraction have been investigated for the determination of bismuth and tellurium in yogurt by hydride generation atomic fluorescence spectrometry.

Results: Highest levels of Bi and Te found in a variety of commercially available samples were 10.0 ± 0.8 ng mL^{-1} and 0.91 ± 0.05 ng mL^{-1}, respectively. These results are on the same order or at lower concentration than in milk indicating that these two elements are not modified in a significant way throughout the technological process involved in yogurt manufacture.

Conclusion: All three methods gave coincident results in samples elaborated with skimmed or partially skimmed milk, nevertheless sonication gave lower values for samples with major fat content. The use of microwave-assisted extraction is recommended because it offers a less time consuming methodology.

Keywords: Atomic fluorescence spectrometry, bismuth, food, hydride generation, metals, microwave digestion, microwave extraction, tellurium, ultrasound extraction, yogurt.

1. INTRODUCTION

Tellurium is a non-essential toxic element widely used in the industry and agriculture fields. Its use also emerged in various innovative materials such as CdTe quantum dots and telluride nanoparticles, clusters and nanotubes. As during the last decades Te based products make their way into landfills, human exposure has increased and because of that, analytical methods are required for its control in foods and wastes. Te toxicity is produced because of its analogy with selenium compounds; tellurium compounds can react with thiols groups from biologically important molecules and inhibits their activities [1, 2].

Bismuth is a heavy metal used in metallurgy, cosmetics and pharmaceuticals. Because Bi compounds are poorly absorbed this element is considered of low toxicity but there is still limited information about Bi uptake, transport and metabolism [1].

Dairy products are rich in nutrients that are essential for a high bone mineral density [3]. Furthermore, several studies relate additional health benefits to the regular consumption of fermented milk products such as yogurt [3-5]. In comparison with cow milk, mineral composition of yogurts is affected by manufacture practices and thus the analysis of mineral elements in yogurt samples requires specific analytical methodologies [6-8].

In recent literature, inductively coupled plasma optical emission spectroscopy (ICP-OES) [8-11], inductively coupled plasma mass spectrometry (ICP-MS) [12, 13], atomic absorption spectroscopy (AAS) [6, 14, 15], and bioassay methodology [16] have been employed to evaluate the presence of mineral elements in dairy products including yogurts. However, Te and Bi have been determined in milk samples [17-21] but, in our knowledge, there is not available information about Te and Bi determination in yogurt samples.

The common characteristic of Bi and Te of forming volatile covalent hydrides allows us the selective separation of these elements from matrices and their complete transfer to the detector for atomic spectroscopy determination. So, hydride generation coupled to non-dispersive atomic fluorescence spectroscopy (HG-AFS) makes possible the analysis of Bi and Te with a relatively high sensitivity and low-cost in a simple way after sample treatment [22].

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Acid microwave-assisted digestion has been demonstrated to be effective for the determination of the total mineral content of yogurt samples [9, 13, 15]. Furthermore, according to Srogi [23], in environmental samples, microwave-assisted extraction with aqua regia turned out to be superior when compared with an analogous nitric acid procedure and EPA method 3051.

On the other hand, following our own experience in the analysis of mineral elements in milk, slurry sampling through ultrasound-assisted extraction with aqua regia could be appropriate for yogurt pretreatment [17].

Therefore, the goal of this work has been the evaluation of three sample preparation approaches, including microwave-assisted digestion, microwave-assisted extraction and ultrasound-assisted extraction for the determination of bismuth and tellurium by HG-AFS in yogurt samples and also to provide, for the first time, data about the content of these two elements in yogurt.

2. MATERIALS AND METHODS

2.1. Instruments and Reagents

A continuous flow hydride generation atomic fluorescence spectrometer model PS Analytical Millenium Excalibur 10055 from PS Analytical (Orpington, UK) was employed for Bi and Te detection. The system was equipped with boosted discharge hollow cathode lamps for Bi and Te from Photron (Victoria, Australia), a specific filter, a solar blind detector and a Perma Pure dryer.

A microwave laboratory system Ethos SEL from Milestone (Sorisole, Italy) equipped with a fibre optic sensor for automatic temperature control, an automatic gas detector and ten high pressure vessels of 100 mL inner volume, operating at a maximum exit power of 1000 W was employed for microwave-assisted digestions and microwave-assisted extractions. An ultrasound water bath Selecta (Barcelona, Spain) operating at 50 W and 50 Hz frequency was employed for sample sonication. A water bath model Tectron 200 from Selecta was also used.

All reagents used were of analytical grade and all solutions were prepared in ultrapure water, with a minimum resistivity of 18.2 MΩ cm, obtained from a Milli-Q system Millipore (Bedford, USA).

Stock solutions of 1000 mg L^{-1} Bi(III) and Te (IV) were prepared by dissolving the appropriate amount of $Bi(NO_3)_3 \cdot 5H_2O$ from Scharlau (Barcelona, Spain) and Na_2TeO_3 from Aldrich Chemicals (Milwaukee, WI, USA), respectively. Sodium tetrahydroborate solution, employed to generate the covalent hydrides, was daily prepared from the corresponding solid product from Fluka (Buchs, Switzerland) dissolved in NaOH 0.1 mol L^{-1} from Probus (Barcelona, Spain), and filtered through a 0.45 μm nylon membrane from Lida (Kenosha, Wisconsin, USA).

For sample treatment and sample dilution, HNO_3 69 % (w/w), H_2O_2 35 % (w/w) and HCl 37 % (w/w) from Scharlau were used. Aqua regia was prepared by mixing HNO_3 and HCl 1:3 (v/v). KBr 1 % (w/v) was prepared from the corresponding salt from Merck (Darmstadt, Germany). A surfactant agent Triton XT-114 Fenbiochimica (Heidelberg, Germany) 0.4 % (v/v) and Antifoam A 2.5 % (w/v) from Sigma (Steinheim, Germany) were also employed.

Argon C-45 (purity higher than 99.995 %), supplied by Carburos Metálicos (Barcelona, Spain) was used as carrier and drier gas in the HG-AFS system.

2.2. General Procedures

2.2.1. Microwave-assisted Digestion

1.00 ± 0.01 g of yogurt was accurately weighed inside pre-cleaned Teflon vessels, then 8 mL of HNO_3 and 2 mL of H_2O_2 were carefully added. The vessels were closed and placed inside the microwave oven. EPA-3051 temperature programme was applied, from 21°C to 175°C in 5 minutes and irradiation at 175°C during additional 5 minutes.

2.2.2. Microwave-assisted Extraction

2.00 ± 0.01 g of yogurt were accurately weighed inside pre-cleaned Teflon vessels and 10 mL of aqua regia were added. The vessels were closed and placed inside the microwave oven. Samples were irradiated at 70°C for 4 minutes.

2.2.3. Ultrasound-assisted Extraction

2.00 ± 0.01 g of yogurt were weighed inside pre-cleaned vessels and 4 mL of aqua regia were added. Four mL of Triton XT-114 0.4 % (v/v) and few drops of antifoam A 2.5 % (w/v) were added to each vessel and the obtained slurries were sonicated for 10 minutes.

2.2.4. Sample Dilution

After sample digestion or extraction, obtained solutions were allowed to cool, transferred to a volumetric flask, in which it was diluted to 25 or 50 mL. The final medium was fitted to 4 mol L^{-1} HCl. In the case of Te determination, Te(VI) was reduced to Te(IV) with KBr 1 % (w/v) by heating at 60 °C during 30 minutes for total Te determination.

2.2.5. Hydride Generation Atomic Fluorescence Spectroscopy

Instrumental conditions for HG-AFS were adjusted according to previous studies focused to improve the analytical signal and its sensitivity [24, 25]. Table **1** summarizes the experimental conditions employed for Bi and Te determination in yogurt samples. Employed standard solutions were prepared daily by sequential dilution and treated in the same way as diluted samples. Two blank digestions were subjected to the same treatment employed for samples and analysed together for every set of samples. Te and Bi concentrations were determined using external calibration in all the cases.

3. RESULTS AND DISCUSSION

3.1. Determination of Bi in yogurt Samples

Yogurt samples, acquired from local markets, from among those that are widely consumed, namely Greek sugared, Macedonian, Petit style, Natural, Natural sugar free and Chocolate were analyzed. Table **2** shows the data found in these samples obtained by HG-AFS after microwave-assisted

Table 1. Experimental conditions for HG-AFS determination of bismuth and tellurium.

Parameter	Bismuth	Tellurium
Wavelength (nm)	223.1	214.3
Primary current (mA)	12	15
Boost current (mA)	10	17.5
Sample volume (mL)	2	2
Delay time (s)	15	10
Analysis time (s)	30	30
Memory time (s)	30	30
Measurement mode	Peak height	Peak height
HCl (mol L^{-1})	4	4
$NaBH_4$ (% w/v)	1	1.2
Ar flow rate (mL min^{-1})	430	270
Air flow rate (L min^{-1})	2.5	2.5
Carrier flow rate (mL min^{-1})	9	9
$NaBH_4$ flow rate (mL min^{-1})	4.5	4.5
Sample flow rate (mL min^{-1})	9	9
Reaction coil length (cm)	150	150

Table 2. Determination of bismuth (ng g^{-1}) in yogurt samples.

Samples	Microwave-assisted Digestion	Microwave-assisted Extraction	Ultrasound-assisted Extraction
Greek sugared yogurt	1.72 ± 0.11	1.65 ± 0.08	1.01 ± 0.10
Macedonian yogurt	8.1 ± 0.5	7.9 ± 0.3	7.9 ± 0.5
Petit style yogurt	nd	nd	nd
Natural yogurt	10.1 ± 1.0	10.0 ± 0.8	9.7 ± 0.4
Natural sugar free yogurt	1.55 ± 0.06	1.61 ± 0.05	1.64 ± 0.14
Chocolate yogurt	3.31 ± 0.18	3.2 ± 0.3	1.87 ± 0.18

Notes: nd - not detected.

digestion, microwave-assisted extraction, and ultrasound-assisted extraction. Results are expressed in all cases in ng Bi or Te per g^{-1} of yogurt ± the standard deviation corresponding to three independent analyses.

Bismuth was detected at quantitative amounts in all samples except in Petit style yogurt, and values found vary from 1.55 to 10.1 ng g^{-1}. Analysis precision evaluated through relative standard deviation was considered suitable for Bi determination at trace levels because it was lower than 10 % for all considered samples whichever method was employed. All the three sample pretreatment methods assayed gave coincident results in samples elaborated with skimmed or partially skimmed milk; nevertheless sonication provided low values for samples with major fat content such as Greek sugared yogurt and Chocolate yogurt.

Bismuth contents in this study can be compared only with studies performed in milk samples. Comparable results were obtained in a Turkish study employing HG-ICP-OES with values between limit of detection 0.16 ng g^{-1} and 14.5 ng g^{-1} [20]. Also in Brazilian studies employing GF-AAS Bi contents were similar to those obtained by us because it ranged between below a limit of detection of 2.9 ng g^{-1} and 3.2 ng g^{-1} [18, 19]. However, our own studies with cow milk gave high Bi concentrations in the range 11.5 to 27.7 ng g^{-1} [17].

3.2. Determination of Te in Yogurt Samples

Tellurium was found under the limit of detection of 0.006 to 0.8 ng g^{-1} in several samples and a mean value of 0.44 ng g^{-1} as it can be seen in Table **3**. Analysis precision, evaluated

Table 3. Determination of tellurium (ng g^{-1}) in yogurt samples.

Samples	Microwave-assisted Digestion	Microwave-assisted Extraction	Ultrasound-assisted Extraction
Greek sugared yogurt	nd	nd	nd
Macedonian yogurt	0.82 ± 0.06	0.91 ± 0.05	0.96 ± 0.08
Petit style yogurt	nd	nd	0.010 ± 0.003
Natural yogurt	0.66 ± 0.04	0.67 ± 0.02	0.71± 0.08
Natural sugar free yogurt	0.032 ± 0.003	nd	nd
Chocolate yogurt	0.250 ± 0.007	0.210 ± 0.009	nd

Notes: nd - not detected.

through relative standard deviation, was considered suitable for Te analysis at trace level because it was lower than 10 % for all samples. However significant differences were not found for the concentration of Te as a function of the employed sample treatment, with exception of chocolate yogurt.

As compared with previous studies made on Te determination in dairy products, in a 2012 French study employing ICP-MS, Te concentration was similar to or higher than values found in this study [21]. For example, in milk and ultrafresh dairy products, mostly Te contents in French samples were lower than the limit of quantification of 2 ng g^{-1} and the highest values were found in some cheese samples with a maximum value of 32 ng g^{-1}. Our study on cow milk samples showed levels of Te ranging between 0.9 and 9.4 ng g^{-1} [17], higher than the values found in yogurt.

On comparing data reported in Tables **2** and **3** for Bi and Te determination as a function of the sample treatment applied, it can be concluded that the regression line between Bi determination by microwave-assisted extraction (y) and microwave-assisted digestion (x) found an equation: $y = 0.984x - 0.02$ with a R^2 value of 0.9997. This provides t values of 1.95 for the comparison of the slope with the theoretical value of 1 and a t value of 0.05 for the comparison of the intercept with 0 value, both lower than the theoretical t value of 2.78 established for a probability level of 95% and 4 degrees of freedom, thus indicating the comparability between values found by both methodologies.

In the case of the comparison between ultrasound-assisted extraction (y) and microwave-assisted digestion (x), a regression line of $y = 0.996x - 0.427$ with $R^2 = 0.98$ was found. However, as it can be seen in Table **2** lower values were found for samples produced from fat milk and because of that the ultrasound-assisted methodology cannot be recommended.

Regarding Te determination the regression between microwave-assisted extraction (y) and microwave-assisted digestion (x) provided an equation: $y = 1.091x - 0.021$ with $R^2 = 0.994$ and t values for the Student test applied to the slope and the intercept of 2.11 and 1.09, both lower than 2.78. This indicates good agreement between both series of obtained data which provided a good accuracy. However, the regression between results found after ultrasound-assisted extraction (y) microwave-assisted digestion (x) provided an equation: $y = 1.138x - 0.003$ with $R^2 = 0.998$ and a t slope value of 4.83 which evidenced a systematic error of values found by ultrasound-assisted extraction which are generally higher than those found by using microwave-assisted.

So it can be concluded that microwave sample treatments are the best alternative for the preparation of yogurt samples to determine Bi and Te by HG-AFS, offering the soft extraction with aqua regia inside the microwave oven, an accurate and fast alternative to the complete digestion of samples using HNO_3 and H_2O_2.

3.3. Green Evaluation of Methods Assayed

AFS is a highly sensitive and selective technique. However the use of $NaBH_4$ (4.5 mL min^{-1} of concentrations of the order of 1% (w/v)) and 4 M HCl provided deleterious environmental side effects. So, the injection of a discrete volume of 2 mL sample and the passivation of residues is recommended to reduce the environmental risks as much as possible.

On considering the three sample treatments assayed, the use of 4 mL aqua regia and 10 min sonication at room temperature is that which minimizes the energy and acids consume.

However, the microwave-assisted digestion, with 8 mL HNO_3 and 2 mL H_2O_2, for a total time of 10 min is the procedure, which permits an enhanced accuracy, and the fact that sample treatment is made in closed reactors strongly diminishes the side effects of this step.

4. CONCLUSION

Slurry sampling through ultrasound-assisted extraction with aqua regia which proved to be an accurate treatment for the determination of Bi and Te in cow milk creates difficulties in the analysis of Te providing excess errors and in the case of Bi determination after ultrasound treatment, the extraction remains partially retained in the solid in the case of samples with a high content of fat. However, microwave-assisted procedures are appropriate for yogurt treatment. Digestion with nitric acid and EPA method 3051 was used as reference procedure and results found correspond well with those concerns. So we propose the use of microwave-assisted extraction as a rapid extraction procedure for the determination of Bi and Te in yogurt.

On the other hand, this study provides new data about the presence of Bi and Te in dairy products. In comparison with milk, Bi and Te in yogurts is on the same order or at lower

concentration than in milk indicating that these two elements are not modified in a significant way throughout the technological process involved in yogurt manufacture.

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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ABBREVIATIONS

AAS	=	Atomic Absorption Spectroscopy
EPA	=	Environmental Protection Agency
GF-AAS	=	Graphite Furnace Atomic Absorption Spectrometry
HG-AFS	=	Hydride Generation Atomic Fluorescence Spectrometry
HG-ICP-OES	=	Hydride Generation and Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-MS	=	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	=	Inductively Coupled Plasma Optical Emission Spectroscopy

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3.3. Extracción rápida de las formas tóxicas de arsénico en carne

Fast extraction methodologies for the determination of toxic arsenic in meat

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Fast extraction methodologies for the determination of toxic arsenic in meat

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Summary

A non-chromatographic analytical procedure has been developed for the determination of arsenic in meat samples including the major toxic arsenic species arsenite, arsenate, monomethylarsonic acid (MMA) and dimetylarsinic acid (DMA). The method is based on the extraction of arsenic species in mild conditions, selective trivalent hydride formation and final determination by hydride generation atomic fluorescence spectroscopy (HG-AFS). Different extractant agents and two different procedures, microwave-assisted extraction (MAE) and ultrasound assisted extration at room temperature, were evaluated for As species extraction. The method provided a limit of detection of 0.013 ng mL-1 and a mean relative standard deviation in actual samples of 6.3 %. Data found for toxic As in meat samples varied from 0.42 ng g-1 in chicken muscle to 68 ng g-1 in pork liver. Percentage of toxic As respect to total As varied in the 9-46 % range depending on the meat product.

Keywords: non-chromatographic; toxic arsenic; atomic fluorescence spectroscopy; meat; microwave assisted extraction

Introduction

The main pathway to incorporate arsenic (As) into the organism is through food and drinking water (EFSA, 2014). There are more than 20 As species which differ extensively in their toxicity. Therefore As speciation in food is crucial in understanding potential risk for humans. In general, inorganic As compounds, arsenite, arsenate, and metabolites MMA and DMA, are more toxic than organic ones such as arsenobetaine, arsenocholine and other arsenosugars. Once ingested arsenate easily reduces to arsenite, and arsenite is involved in a series of reductions and oxidative methylations to form MMA and DMA. The Tolerable Weekly Intake for inorganic As 15 μg kg^{-1} body weight is under revision because epidemiological studies had shown that it is no longer appropriate. Maximum levels of 0.1-0.30 mg kg^{-1} fresh weight have been set only for rice products and will be extended to other food when reliable analytical methods became available (EU, 2015).

The highest concentrations of As have been reported in fish, seafood, seaweed and cereals (JECFA, 2011). In marine animals As is predominantly arsenobetaine which is rapidly excreted unchanged whereas seaweed and rice present high proportions of inorganic As (Lynch et al., 2014, Ruiz-de-Cenzano et al., 2015). Therefore, progress in the assessment of total dietary intake of As requires to extend the aforementioned studies to other components of the diet such as meat products (EU, 2015, Lynch, 2014). It is important quantifying arsenic in different animal species due to variations regarding factors such as feeding practices, geographical conditions and metabolic patterns amongst others. Furthermore analysis should include not only muscle but also internal organs such as liver, heart or kidney which are consumed too.

Arsenic may reach meat by two main sources: i) trough plants growing in naturally As-rich soils or industrially polluted with As soils, ii) through organoarsenicals drugs used in poultry production (Pussa, 2013). These organoarsenicals, which have low toxicity to animals, can be metabolized to the more toxic As(III) and As(V) (Zhang, 2015, Conklin, 2012, Liu, 2016) and through repeated annual poultry litter applications to agricultural soils may result in arsenic buildup in soil and lead to plant uptake and subsequent transfer to the human food chain (Cui, 2013).

Organoarsenicals, roxarsone, arsanilic acid, nitarsone and carbasone have been widely used as feed additives in the poultry industry. The European Union (EC, 1999) and the United States

(US FDA, 2015) have ceased the use but continue to be legally used in many other countries (Liu, 2016).

To date, research on arsenic concentration in different animal species have primarily focused on liver and kidney which typically accumulate higher concentrations (López-Alonso et al., 2016; Liu, 2016, Ortega-Barrales, 2015), or have been focused on the controversial use of organoarsenicals (Lynch et al., 2014). There is a notable absence of research on arsenic in muscle tissue (López-Alonso et al., 2016) and research on toxic species. Accurate identification and quantification As species in meat is a challenge due to their low concentrations (Liu, 2016). Püssa reviewed that inorganic arsenic present in meat accounts for the 75 % of the total As in red meats and 65% in poultry (Püssa, 2013). In contrast, Ortega-Barrales reported in a 2015 publication that arsenic present in meat was predominantly in organic forms, DMA together with arsenobetaine and minor amounts of MMA (Ortega-Barrales, Fernández-de Córdova, 2015).

For arsenic speciation in solid foods as meat, the extraction procedure must be performed under mild conditions in order to avoid the interconversion of species. Moreover low levels of As species requires low limits of detection on the determination method thus high sensitivity techniques. Analytical methods recently employed in arsenic speciation in meat samples involves traditional liquid-liquid extraction (Conklin et al., 2012), ultrasounds extraction (Monasterio et al., 2011; Batista et al., 2012; Liu et al., 2013; Guo et al., 2013), accelerated solvent extraction (Cui et al., 2013), and microwave assisted extraction (Zhang et al., 2015), in combination with chromatographic separation of species. Liquid chromatography coupled to HG-AFS (Monasterio et al., 2011; Cui et al., 2013) and mostly inductively coupled plasma mass spectrometry (ICP-MS) (Batista et al., 2012; Guo et al., 2013; Zhang et al., 2015; Fontcuberta et al., 2011; Yang et al., 2011; Mao et al., 2011; Peng et al., 2014; Liu et al., 2016) and ion chromatography coupled to ICP-MS (Conklin et al., 2012), and capillary electrophoresis coupled to ICP-MS (Liu et al., 2013) have been employed for As speciation. However non-chromatographic speciation approaches are not extended in As speciation in meat. In a literature revision we only found one work performed by Schoof et al., who determine As(III), As(V), DMA and MMA by hydride generation atomic absorption spectroscopy using cryogenic trapping of hydrides which show different boiling points (Schoof et al., 1999).

Non-chromatographic methodologies let to obtain enough information in many situations for example to difference between toxic and non-toxic species, with the advantages of being

economic and less time consuming than chromatographic ones (Gonzalvez et al., 2010). In this work it has been developed a non-chromatographic approach for the determination of toxic arsenic species (arsenite, arsenate, MMA and DMA) from different meat products. It combines extraction of species in mild conditions with the excellent sensitivity of atomic fluorescence and selectivity of hydride generation. Two alternatives for species extraction were evaluated, the use of MAE and ultrasound assisted extraction at room temperature. The selected method was evaluated in front of results provided by reference microwave EPA method. Additionally, total As data were obtained from a proposed total As determination method involving HG-AFS, which was evaluated in front of results provided by reference ICP-MS method.

Experimental

Apparatus and reagents

A continuous flow hydride generation atomic fluorescence spectrometer PSA Millenium Excalibur 10055 from PS Analytical (Orpington, United Kingdom) working in Table S1 conditions and an inductively coupled plasma mass spectrometer Perkin Elmer Elan 6000 **(**Thornhill, Canada) were employed for As determination.

Table S1

Wavelength (nm)	197.3
Primary current (mA)	27.5
Boost current (mA)	35
Sample volume (mL)	2
Delay time (s)	10
Analysis time (s)	30
Memory time (s)	30
Filter	32
HCl (mol L^{-1})	3.5
$NaBH_4$ % (m/v)	1
Argon flow rate (mL min^{-1})	330
Air flow rate (L min^{-1})	2.5
Carrier flow rate (mL min^{-1})	9
$NaBH_4$ flow rate (mL min^{-1})	4.5
Reaction coil length (cm)	150

A microwave laboratory system Ethos SEL from Milestone (Sorisole, Italy) and an ultrasound water bath from Selecta (Barcelona, Spain) were employed for microwave-assisted digestion and for microwave-and ultrasound assisted extraction of As.

Lyophilisation of samples was carried out using a freeze drier system Cryodos 50 from Telstar (Barcelona, Spain).

All reagents used were of the highest purity and all solutions were prepared in ultrapure water, obtained from a Milli-Q system Millipore (Bedford, USA) with a resistivity of 18.2 MΩcm.

As(III) stock standard of 1000 mg L^{-1} was prepared by dissolving As_2O_3 from Riedel de Häen (Hannover, Germany) as described in a previous work (Cava-Montesinos et al., 2005).
Sodium tetrahydroborate solution was prepared from the corresponding salt from Fluka (Buchs, Switzerland) dissolved in NaOH 0.1 mol L^{-1} from Probus (Barcelona, Spain). Antifoam A from Sigma-Aldrich (Steinheim, Germany) was used to avoid foam formation during hydride generation.

As extractant agents were tested HCl 6 mol L^{-1}, prepared from concentrated HCl 37% Scharlau (Barcelona, Spain); HNO_3 1 mol L^{-1} prepared from concentrated HNO_3 70% J.T. Baker (Deventer, USA); aqua regia 10% (v/v) prepared by mixing 2.5 mL concentrated HNO_3 and 7.5 mL concentrated HCl and diluting with ultrapure water to 100 mL; acetic acid 1 mol L^{-1} prepared from the concentrated one from Fluka; H_3PO_4 1 mol L^{-1} from Analar (United Kingdom), MeOH:H_2O 1:1 from suprapure methanol of Scharlau. Triton XT-114 from Feinbiochimica (Heidelberg, Germany), L-cysteine from Sigma-Aldrich, H_2O_2 35% from Scharlau and H_2SO_4 from Panreac were also employed in sample preparation. Synthetic air and high purity argon C-45 (99.995%) from Carburos Metálicos (Barcelona, Spain) were used in HG-AFS.

Samples

13 animal tissue samples were purchased on the local market, including muscle and internal organs.

Samples were cut in small pieces with a plastic knife and frozen at -30 ºC. Afterwards, they were freeze-dried for a minimum of 48 hours at a chamber pressure of 0.05 mBar. The dried

samples were crumbled and pulverized with a domestic mill 160 W Fagor (Mondragon, Spain). The resulting fine powder was stored in a desiccator until analysis.

A certified reference material CRM DORM-2 (dogfish muscle) was obtained from the National Research Council of Canada, Institute of Environmental Chemistry (Ottawa, Canada).

General procedures

Microwave-assisted extraction procedure for toxic arsenic

0.5 g powdered sample were treated with 10 mL of HNO_3 1 mol L^{-1}in microwave labsystem (1 min to reach 70 ºC, 4 min at 70 ºC and 10 min cool down to 35 ºC). Digested samples were treated with 7.3 mL of concentrated HCl and 0.2 mL of 25 % (w/v) L-cysteine and diluted to 25 mL. Then they were heated 1 minute at 100 ºC in a water bath.

Ultrasound-assisted extraction procedure for toxic arsenic

0.5 g powdered sample were suspended in 4 mL of extractant reagent, 4 mL Triton XT-114 0.4% and 250 µL of antifoam A. They were shaken vigorously and the mixture was sonicated 10, 20 or 30 minutes. To the resulting slurries 14.5 mL of concentrated HCl, 2 mL hydroxylamine hydrochloride 25% (w/v) and 1 mL KI 50% were added, the mixture was diluted to 50 mL.

Reference EPA method for toxic arsenic determination

0.5 g powdered sample were treated as described in a previous work (Cava-Montesinos et al., 2005).

Microwave-assisted digestion for total arsenic determination through HG-AFS

0.5 g powdered sample were treated as described in a previous work (Cava-Montesinos et al., 2005).

Reference microwave-assisted digestion for total arsenic determination through ICP-MS

1 g powdered sample was digested in microwave following EPA-3051 method (US EPA, 2007) with 8 mL of concentrated HNO_3 and 2 mL H_2O_2 35 %.

Results and discussion

Selection of extractant agent for extraction of toxic As from meat samples

Arsenic speciation requires the development of soft digestion procedures to extract the target species that not alters species.

The extractant capability of different reagents was evaluated in the CRM DORM-2 certified sample using a microwave-assisted method and an ultrasound-assisted method. In the CRM DORM-2 only total As and arsenobetaine are certified but concentrations for other species are documented in the literature (Cava-Montesinos et al., 2005, Moreda-Piñeiro et al., 2010; Moreda-Piñeiro et al., 2011). The sum of As(III), As(V), MMA and DMA (1.06 $\mu g\ g^{-1}$) was employed as a target value for toxic As.

For microwave-assisted extraction of toxic As, different time (2, 4, 6 min) and temperature (70, 75, 80ºC) of microwave irradiation were tested. Results, in Table 1, show acceptable recoveries for HNO_3 1 mol L^{-1}, aqua regia 10% (v/v) and H_3PO_4 1 mol L^{-1} but aqua regia gives recoveries higher than 100 % probably because it is a hard treatment and produces a partial decomposition of organic species. It must be noticed that an increment on microwave time or temperature does not affect As extraction from meat tissues. Then HNO_3 1 mol L^{-1}, 70 ºC and 4 minutes were considered the best conditions in order to obtain quantitative and reproducible results for the determination of toxic As species in meat products.

In ultrasound-assisted extraction of toxic As, different time of sonication (10, 20 or 30 minutes) was tested. Results shown in Table 1 let to conclude that MeOH:H_2O is unable to quantitatively extract As toxic species from CRM tissue, recovery about 20 % indicates that probably it extracts organic As species but not inorganic ones whereas aqua regia and HNO_3 1 mol L^{-1} provides the best results. Furthermore sonication time strongly affects yield of extraction being 10 minutes insufficient to have quantitative extractions.

Comparable results were obtained by employing both extraction methods. Both procedures are relatively economic and allow processing at least 10 samples simultaneously per run. However MAE involves 10-15 min and ultrasound 20-30 minutes. Furthermore slurry samples produced from ultrasound treatment must to be filtered before to be introduced in the HG-AFS because solid particles could obtrude the system. Therefore MAE was shown to be the mildest,

fastest, least complex and most reproducible of the extraction techniques evaluated in this study for toxic arsenic determination.

Table 1
Recovery of toxic As from SRM DORM-2

Extractant conditions	microwave conditions					ultrasounds conditions		
	2 min 70 ºC	4 min 70 ºC	6 min 70 ºC	2 min 75 ºC	2 min 80 ºC	10 min	20 min	30 min
HCl 6 mol L^{-1}	46 ± 9	43 ± 7	52 ± 8	40 ± 7	49 ± 10	23 ± 5	60 ± 5	51 ± 7
HNO_3 1 mol L^{-1}	90 ± 5	94 ± 4	97 ± 3	91 ± 2	88 ± 7	48 ± 4	87 ± 3	88 ± 9
aqua regia 10%	112 ± 8	117 ± 8	122 ± 9	108 ± 5	115 ± 6	56 ± 8	89 ± 5	85 ± 6
CH_3COOH 1 mol L^{-1}	66 ± 4	72 ± 5	39 ± 6	61 ± 8	66 ± 3	32 ± 4	60 ± 3	63 ± 4
H_3PO_4 1 mol L^{-1}	88 ± 5	88 ± 3	86 ± 4	86 ± 3	90 ± 10	38 ± 6	78 ± 7	68 ± 5
MeOH:H_2O 1:1	23 ± 6	18 ± 3	23 ± 2	18 ± 4	20 ± 6	12 ± 6	17 ± 2	22 ± 4

Average ± standard deviation corresponding to 3 replicates.
Toxic As determined by HG-AFS after microwave-assisted extraction or ultrasounds assisted extraction and treatment with KI in acid medium.
Recovery was calculated from the difference in percentage between the As concentration found and the target value of 1.06 µg g^{-1} established by Cava-Montesinos and others.

Total As in meat

The determination of total As by HG-AFS needs a previous degradation of arsenobetaine. Treatment with H_2SO_4 and HNO_3 at 180-200 ºC allows matrix destruction and organic species degradation into inorganics ones.

Data of sample analysis through the developed methodology were compared with those obtained through a reference ICP-MS procedure (Table 2). There is a clear correlation between data found for total As determined by HG-AFS (y) and data obtained by ICP-MS (x), being the correlation equation $y = (1.022 \pm 0.005)\, x - (0.3 \pm 0.3)$, with $R^2 = 0.9997$. Paired sample test for a probability level of 95% and 12 freedom degrees show that both methodologies are comparable because experimental Student´s t-value (1.52) was lower than the theoretical one (2.18).

Methods for total As determination in meat have been developed in past years and consequently application studies began to be regularly performed in order to increase databases (JECFA, 2011; Bilandžić et al., 2010; Blanco-Penedo et al., 2010; Hassan et al., 2012; Chen et al., 2011; Lei et al., 2013; Hwang et al., 2011; Wang et al., 2013; Alkmim Filho et al., 2014; Ghosh et al., 2012; Roggeman et al., 2014; Bortey-Sam et al., 2015).

In present study, obtained data for the analysis of total As in meat are in the same order of those obtained in unpolluted areas by other authors. Total arsenic found in muscle samples ranged between 2.6 ng g^{-1} dry weight (dw) in chicken and 25.4 ng g^{-1} dw in ox that represent near 0.7-6.1 ng g^{-1} expressed as fresh weight (fw). Total arsenic found in internal organs showed a high variability because liver is a detoxificant organ and accumulates transition elements as metal and metalloids, it varied from 2.03 ng g^{-1} (0.7 ng g^{-1} fw) to 170 ng g^{-1} (51 ng g^{-1} fw).

Table 2
Total As in meat samples

Sample	t-As (ng g^{-1} dry weight)		t-As (ng g^{-1} fresh weight)
	Proposed HG-AFS	Reference ICP-MS	
cow muscle	18.2 ± 1.0	18.4 ± 1.7	6.7
veal muscle	11.3 ± 0.9	11.5 ± 0.6	3.3
ox muscle	25.4 ± 0.9	26.5 ± 1.5	6.1
chicken muscle	2.60 ± 0.10	2.69 ± 0.02	0.7
lamb muscle	6.3 ± 0.3	6.30 ± 0.10	2.2
rabbit liver	120 ± 4	120 ± 4	42.2
pork liver	170 ± 7	162.4 ± 0.4	51.1
chicken liver	2.03 ± 0.18	1.88 ± 0.06	0.7
veal liver	23.5 ± 1.3	22.1 ± 0.5	9.3
lamb liver	120 ± 8	120 ± 3	44.9
veal kidney	5.7 ± 1.5	5.30 ± 0.10	2.2
lamb kidney	30.6 ± 1.4	31.7 ± 0.8	8.1
chicken heart	0.200 ± 0.010	0.17 ± 0.02	0.1

Average ± standard deviation of 3 replicates. Total As expressed as ng g^{-1} wet weight was estimated from water content determination and total As determined in dry samples.

Toxic As in meat

Treatment with L-cysteine allows final conversion of As(V) into As(III) which is the most sensible specie in HG-AFS. Other species present in the sample extract, such as arsenobetaine, do not react with $NaBH_4$ to form the corresponding hydride. Those, it is possible to perform a selective determination of toxic As.

Determination of toxic As in meat was performed employing external calibration curve. Calibration standards were prepared in a calibration range up to 2 ng mL^{-1} in the same conditions as sample solutions, with HCl 3.5 M and L-cysteine 0.2 % (w/v). Analytical figures of merit obtained for the developed procedure concern a sensitivity of 1896 fluorescence units per ng mL^{-1} As, with a good linearity (R^2 = 0.9997) The limit of detection calculated as the concentration corresponding to signals equal to three times the standard deviation of ten reagents blank

measurements was 0.013 ng mL^{-1}. In short, all these parameters are adequate for the determination of trace As in meat products.

Results of toxic As determination in samples are shown in Table 3 and compared with those employing reference EPA method. There is a clear correlation between data found from developed method (y) and data obtained by EPA method (x), being the correlation equation $y = (0.990 \pm 0.013)\, x - (0.3 \pm 0.3)$, with $R^2 = 0.9998$. Paired sample test for a probability level of 95% and 12 freedom degrees show that both methodologies are comparable because experimental Student´s t-value (1.63) was lower than the theoretical one (2.18).

Table 3
Toxic As in meat samples

Sample	Toxic As (ng g^{-1} dry weight)		Toxic As (%)
	Proposed MAE method	Reference EPA method	
cow muscle	3.1 ± 0.3	3.01 ± 0.19	17
veal muscle	1.47 ± 0.10	1.42 ± 0.08	13
ox muscle	5.9 ± 0.2	5.9 ± 0.2	23
chicken muscle	0.42 ± 0.02	0.32 ± 0.03	16
lamb muscle	1.36 ± 0.10	1.35 ± 0.10	22
rabbit liver	42 ± 2	43.0 ± 1.6	34
pork liver	68 ± 5	68 ± 3	41
chicken liver	0.94 ± 0.10	1.02 ± 0.07	46
veal liver	4.3 ± 0.2	4.8 ± 0.3	18
lamb liver	23.3 ± 0.6	25.5 ± 0.9	19
veal kidney	0.52 ± 0.07	0.64 ± 0.10	9
lamb kidney	10.9 ± 0.4	13.3 ± 0.5	36
chicken heart	nd	nd	-

Average ± standard deviation corresponding to 3 replicates. nd= not detected
Toxic As expressed as percentage was estimated from total As (Table 2) and toxic arsenic determined by proposed MAE method.

Data on As species in meat are scarce. Most papers are focused on developing suitable analytical methodologies for As speciation in meat and use few samples or fortified ones. Additionally most of them deal with As organic species but are not sensitive enough to determine toxic inorganic As species. In Table 4 are summarized published papers on toxic inorganic As species reviewed from 2010 to 2016. DMA is generally the predominant toxic As form followed by MMA, As(V) and As(III).

In this work, quantification of toxic arsenic in 12 of 13 samples analysed provided results which varied from 0.42 ng g^{-1} in chicken muscle to 68 ng g^{-1} in pork liver. Percentage of toxic As

respect to total As varied in the 9-46 % range depending on the meat product thus makes difficult to extract a general conclusion about organic or inorganic As predominance in meat.

Table 4
Speciation data of toxic arsenic in meat products published in the scientific literature

Sample	n	t-iAs ng g^{-1}	As(III) ng g^{-1}	As(V) ng g^{-1}	DMA ng g^{-1}	MMA ng g^{-1}	Reference
chicken muscle	3	--	--	nd-21	--	--	(Guo et al., 2013)
chicken muscle	1	57	--	--	65	nd	(Mao et al., 2011)
chicken muscle	1	41	--	--	18	nd	(Zhang et al., 2015)
chicken muscle	1	--	458	nd	nd	nd	(Liu et al., 2013)
chicken muscle	1	--	6	17	27	41	(Batista et al., 2012)
bovine muscle	1	--	nd	15	9	7	(Batista et al., 2012)
chicken liver	1	35	--	--	108	nd	(Mao et al., 2011)
chicken liver	8	--	9.3	4.5	12	1.2	(Peng et al., 2014)
chicken liver	1	--	--	--	15.7*	nd	(Monasterio et al., 2011)
chicken liver	6	nd-1177	--	--	nd-104	nd-135	(Zhang et al., 2015)
chicken gizzard	1	175	--	--	23	nd	(Zhang et al., 2015)

* µg L^{-1}
. nd= not detected

Conclusions

In this study a fast, simple and acurate methology was developed for determination of toxic species. Chromatographic separation is not required because only toxic species derived from arsenite, arsenate, MMA and DMA form the corresponding hydrides in reaction with $NaBH_4$ and they are detected through HG-AFS. Different types of meat products were analized in order to verify the applicability for pratical analysis. The accuracy of the method was assured by comparing results found with those obtained from reference method. Low limit of detection and good linearity makes this method able to quantify toxic As at difference levels in different kind of meat products.

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Conflict of interest

The authors declare that there is no conflict of interest.

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