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Technical note

Oxygen bomb combustion of biological samples for inductively coupled plasma optical emission spectrometry[☆]

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Abstract

A rapid sample preparation method is proposed for decomposition of milk powder, corn bran, bovine and fish tissues, containing certified contents of the analytes. The procedure involves sample combustion in a commercial stainless steel oxygen bomb operating at 25 bar. Most of the samples were decomposed within 5 min. Diluted nitric acid or water-soluble tertiary amines 10% v/v were used as absorption solutions. Calcium, Cu, K, Mg, Na, P, S and Zn were recovered with the bomb washings and determined by inductively coupled plasma optical emission spectrometry (ICP-OES). Ethanol mixed with paraffin was used as a combustion aid to allow complete combustion. A cooling step prior releasing of the bomb valve was employed to increase the efficiency of sample combustion. Iodine was also determined in milk samples spiked with potassium iodide to evaluate the volatilization and collection of iodine in amine CFA-C medium and the feasibility of its determination by ICP-OES with axial view configuration. Most of the element recoveries in the samples were between 91 and 105% and the certified and found contents exhibited a fair agreement at a 95% confidence level.

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1. Introduction

The use of an elevated pressure of oxygen inside a closed metal container in the form of oxygen bomb combustion is an alternative procedure for complete oxidation of biological samples. Combustion with oxygen in sealed bomb has been used to convert solid and liquid combustible samples into soluble forms for chemical analysis. In this system, the organic matter is oxidized to carbon dioxide and water by the combustion reaction, and the volatile components, formed by burning, are trapped in an absorption solution [1]. Hicks et al. [2] proposed a rapid determination of sulfur in coal after sample combustion in an oxygen bomb. The sample was burnt using the normal practices involved in determining the calorific value of coal, and sulfur was determined by potentiometric titration. An oxygen combustion bomb-ion chromatography (IC) method has been reported for elemental analysis of organic compounds, fuel, and hazardous wastes with indirect photometric detection [3,4]. Problems facing the combustion procedure were discussed and methods for solving them were evaluated. The reliability of the oxygen bomb IC procedures was established by testing the recoveries of a large group of organic compounds containing various heteroatoms. The procedure was shown to provide a satisfactory method for elemental determination of heteroatoms in organic compounds. The IC was also used to separate halogens, nitrogen, and sulfur in coal and oil shale samples after combustion in a Parr oxygen bomb containing water. Combustion was carried out at 30 bar of oxygen [5]. The authors also proposed the application of IC for determination of sulfur species in wastewater and leachates from coal. In this case, samples were mixed with white oil as a combustion aid, and water. Precision of the method was approximately 5%. Mills [6] studied the volatility behavior of boron during ashing of coal. The procedure developed allowed boron determination after combustion of 1.0 g of whole coal in an oxygen bomb. A 10-min cooling step was used before releasing the bomb pressure. The remaining ashes were acid dissolved, and boron was determined by inductively coupled plasma optical emis-

sion spectrometry (ICP-OES) with conventional glass nebulizer.

The use of a combustion aid may be necessary for an efficient decomposition depending on the sample matrix, its texture (grainy, solid, and fluid), as well as the combustible portions and their gross calorific value. A few combustion aids such as ethanol, paraffin, benzoic acid (tablet form), acetobuturate capsules, and PE/PP balls have proven to be particularly suitable to improve sample decomposition [7,8]. It helps to ensure that the required decomposition temperature (>1000 – 2000 °C) in the sample core and the required burning time are achieved. For samples which have a high inorganic content or in the case of porous, absorbent samples, it is advantageous to mix completely the sample and the combustion aid. Occlusions from surface melting are thus avoided for the most part since the mechanical sample structure is broken down into the smallest parts. Combustion of the sample matrix has to be promoted slowly in order to avoid having portions of the same expelled out of the crucible through sudden, explosive combustion and thus only partly decomposed [9].

The oxygen bomb can also be suitable for total determination of iodine in biological samples due to the volatility of this element. Iodine is known as an essential trace element and plays an important role in metabolic processes in human and animals. However, most of the methods for its determination are tedious, expensive, or requires extensive sample preparation. A few authors showed that combustion in a Schöniger flask or in the Trace-O-Mat[®] oxygen combustion apparatus can be successfully applied for iodine determination [10–12]. They also showed that volatile iodine can be quantitatively recovered in absorbing solution of tetramethylammonium hydroxide [10] or water-soluble tertiary amines, so called CFA-C [11,12]. The collection of iodine in alkaline medium avoids memory effects caused by formation of volatile compounds in acidic medium (HI and I₂) during pneumatic nebulization.

This work aims to evaluate an oxygen combustion procedure for determination of Cu, Ca, K, Mg, Na, P, S and Zn in certified reference material by ICP-OES. It also focus on the determination of

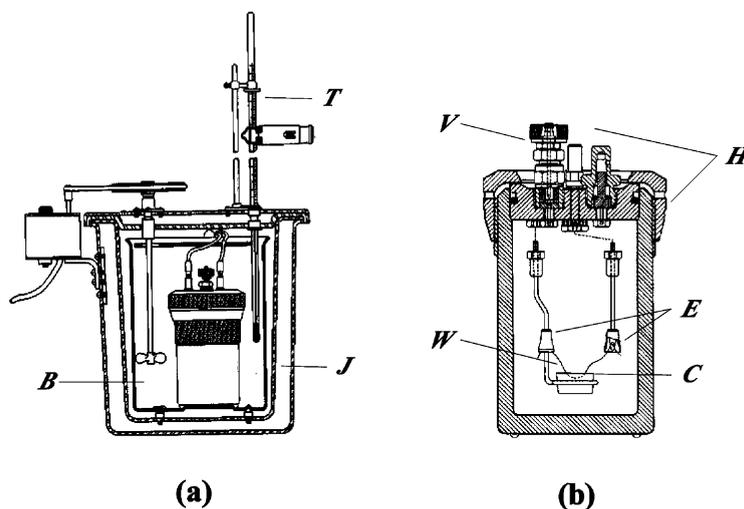


Fig. 1. (a) A cross-section of a self-containing calorimeter: B, oval calorimeter bucket; T, calorimeter thermometer; J, water-jacket; and (b) a 340-ml oxygen bomb including: H, one-piece head with a gas inlet fitting; V, releasing valve; E, quick grip electrodes; W, fuse wire; C, sample capsule. (Adapted from Parr Instruments Operating Instructions, Introduction to Bomb Calorimetry—No 202, Moline, IL, 1978.)

iodine in milk spiked with potassium iodide prior to combustion to evaluate the volatilization and absorption of iodine in amines solution medium, and the feasibility of its determination by ICP-OES axial view configuration.

2. Experimental

2.1. Instrumentation

A self-contained bomb calorimeter with a water-jacketed bucket chamber and automatic temperature controls (Fig. 1a) (Parr Instrument Company, Moline, IL) was used for sample preparation. Sample combustion was carried out in a high pressure stainless steel oxygen bomb (Fig. 1b) with a capacity of 340 ml. The bomb was fired via an electrical discharge through a Ni–Cr fuse wire (Ref. 45C10, Parr Instrument) in contact with the sample (~ 250 mg) placed in a stainless steel capsule. The oxygen pressure inside the bomb was 25 bar. After combustion and cooling step, the gas was carefully released through a gas valve to reach pressure of 1 bar.

A simultaneous ICP-OES with radial view configuration (Varian VISTA, Mulgrave, Australia)

was used for element determination. Nebulization was performed through a concentric nebulizer coupled to a cyclone type nebulization chamber (Glass Expansion, Victory, Australia). Iodine was determined by axial view ICP-OES with a V-groove nebulizer in a Sturman-Masters spray chamber.

2.2. Reagents and samples

Distilled, deionized water of $18\text{ M}\Omega\text{ cm}$ resistivity, obtained from a Milli-Q system (Millipore, Bedford, MD), was used to prepare all solutions. Reagent grade HNO_3 (0.1% v/v) (Carlo Erba, Milan, Italy) was used as absorption solution for the majority of the elements. Aqueous solution (10% v/v) of mixed tertiary amines (CFA-C Reagent, Spectrasol, Warwick, USA) [13] was used as absorption solution for iodine determination. Spex plasma standard (1000 mg l^{-1}) (Metuchen, USA) was used to prepare Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, and Zn reference solutions, which ranged from 2.5 to 100 mg l^{-1} . Reagent grade KI (LabSynth, São Paulo, Brazil) was used to prepare all iodine solutions as well as for milk sample spiking before combustion. Etha-

nol (LabSynth) and paraffin (from local market) were used as combustion aids. All glassware and plasticware was washed with diluted neutral detergent, soaked overnight in 10% v/v HNO₃, and rinsed with deionized water before use.

The samples investigated were certified reference materials from the National Institute of Standards and Technology—NIST (USA): bovine liver—1577b, bovine muscle—8414, corn bran—8433, and whole milk powder 8435, and from the Community Bureau of Reference—BCR (Belgium): cod muscle—422. The fine-grained samples were homogenized to allow good reproducibility of decomposition. Commercial non-certified milk powder was used for iodine analysis.

2.3. Sample combustion procedure

Sample combustion was carried out in a high pressure stainless steel oxygen bomb of a calorimeter system. Approximately 5 ml of nitric acid (0.1% v/v) or 10 ml of amine CFA-C (10% v/v) were placed in the bottom of the combustion bomb to absorb the volatile elements. Ethanol (0.5 ml) mixed with paraffin pellets (35–40 mg) was used as a combustion aid to improve decomposition of the sample. Appropriate energy was introduced to allow good combustion speed. The standard oxygen filling level of the decomposition vessel was 25 bar.

Since the combustion can cause the decomposition vessel to become hot (50–55 °C), the oxygen bomb was submersed in a water bath during combustion for fast release of heat from the vessel, and thus to provide sufficient condensation and absorption of the ions in the absorption solution. Ignition of the sample was carried out by heating and melting down the fuse wire submersed in the sample (~250 mg). No ignition problems were observed. Since the oxygen bomb is a pressure vessel, safety provisions were taken into account. After combustion and cooling steps, the gas was carefully released through a gas valve to reach pressures of approximately 1 bar. Thus, the bomb washings as well as dust-like remaining ashes were transferred to 25-ml flasks and diluted with distilled deionized water. The final digests

Table 1
Operational parameters for ICP-OES measurements

	Radial
Power (kW)	1.3
Plasma gas flow (l min ⁻¹)	15
Auxiliary gas flow (l min ⁻¹)	1.5
Nebulizer gas flow (l min ⁻¹)	0.7
Observation height (mm)	8
Sample uptake rate (ml min ⁻¹)	0.8
Analytical wavelength (nm) ^a	
Ca II	317.938
Cr II	267.716
Cu I	327.395
Fe II	238.205
I ^b I	179.847
K I	766.507
Mg II	280.275
Mn II	257.610
Na I	588.988
Ni II	231.607
P I	177.433
S I	181.972
Zn I	202.549

^a I and II, atomic and ionic emission line, respectively.

^b Determined by axial-viewed ICP-OES.

were acidified to 1% v/v with HNO₃, except for tertiary amines solution used for iodine absorption.

Non-certified milk powder samples (~500 mg) used for iodine analysis were spiked with different amounts of KI and ground for homogenization. These samples were combusted as the procedure described above.

2.4. Element determination by ICP-OES

Table 1 lists the operational parameters of the spectrometer. Three emission lines (atomic and ionic) for each element were tested before selection. The choice of the analytes spectral lines was based on both their sensitivity and spectral interferences. The background correction for the selected analyte lines was the fitted mode in which the peak shaped functions was fitted to the analyte peak. The operational parameters of the axial-viewed ICP-OES for iodine determination were the same as for the radial configuration, except for the nebulizer gas flow rate, which was 0.9 l min⁻¹, and the observation height. Tests were

Table 2
Mineral contents (dry basis) in biological samples determined by ICP-OES after combustion

Sample	Cu ($\mu\text{g g}^{-1}$)	Zn ($\mu\text{g g}^{-1}$)	Ca (mg g^{-1})	K (mg g^{-1})	Mg (mg g^{-1})	Na (mg g^{-1})	P (mg g^{-1})	S (mg g^{-1})
<i>Bovine muscle</i>								
Certified	2.84 ± 0.45	142 ± 14	0.145 ± 0.020	15.2 ± 0.4	0.960 ± 0.095	2.10 ± 0.08	8.36 ± 0.45	7.95 ± 0.41
Found	5.51 ± 2.40	145 ± 7	0.159 ± 0.021	14.9 ± 0.1	0.907 ± 0.010	2.01 ± 0.08	7.90 ± 0.10	7.36 ± 0.53
<i>Bovine liver</i>								
Certified	160 ± 8	127 ± 16	0.116 ± 0.004	9.94 ± 0.02	0.601 ± 0.028	2.42 ± 0.06	11.0 ± 0.3	7.85 ± 0.06
Found	154 ± 10	140 ± 22	0.121 ± 0.006	9.98 ± 0.35	0.589 ± 0.064	2.31 ± 0.11	10.6 ± 0.1	7.03 ± 1.27
<i>Cod muscle^a</i>								
Certified	0.6 ± 0.4	19.6 ± 0.5	0.33^a	21.7^a	1.37^a	2.20^a	N/A	11.5^a
Found	< 6.0	22.1 ± 0.2	0.309 ± 0.031	19.9 ± 1.7	1.32 ± 0.01	2.09 ± 0.16	N/A	10.3 ± 1.0
<i>Corn bran</i>								
Certified	2.47 ± 0.40	18.6 ± 2.2	0.420 ± 0.038	0.566 ± 0.075	0.818 ± 0.059	0.430 ± 0.031	0.171 ± 0.011	0.860 ± 0.150
Found	< 6.0	20.0 ± 0.3	0.340 ± 0.035	0.548 ± 0.034	0.637 ± 0.010	0.415 ± 0.040	0.120 ± 0.035	0.758 ± 0.030
<i>Milk powder</i>								
Certified	0.46 ± 0.08	28.0 ± 3.1	9.22 ± 0.49	13.6 ± 0.5	0.814 ± 0.076	3.56 ± 0.40	7.80 ± 0.49	2.65 ± 0.35
Found	< 6.0	25.1 ± 2.1	8.18 ± 0.12	12.6 ± 0.4	0.734 ± 0.032	3.12 ± 0.21	6.90 ± 0.20	2.44 ± 0.21

Found values: mean \pm S.D. ($n=3$); certified values are given as confidence intervals; N/A, not available.

^a Non-certified values.

performed to evaluate P and S interference due to their emission lines being close to that of iodine.

3. Results and discussion

Table 2 shows the results of the elemental analysis after sample combustion. For the majority of the samples, certified and found contents exhibited a fair agreement at a 95% confidence level. The relative standard deviations for most of the samples were either below or approximately 10%. These results indicate the efficiency of the proposed method.

Comparing to the use of Schöniger combustion flask [10–12], one advantage of employing the combustion bomb is the sample mass. This latter device can work with sample mass as high as 1 g [2], while the Schöniger flask is usually limited to 30–50 mg. This small amount of sample needs a highly homogeneous sample that can be difficult to obtain without a preliminary grinding step.

Except for iodine studies, both amine CFA-C and HNO₃ were efficient as absorption solutions for all elements. Therefore, as iodine is more efficiently absorbed in alkaline solutions, amine CFA-C was chosen for its collection, and HNO₃

0.1% v/v for the remaining elements. Apparently, there was no reaction of nitric acid with the stainless steel vessel and no detectable blank contamination was observed from the Ni–Cr fuse wire, at the levels investigated. All measurements were blank and background corrected. Since, a combustion aid is usually recommended to increase the efficiency of sample combustion [6,7], ethanol and paraffin were used for all materials. The use of a 5–10 min cooling step prior to releasing the bomb valve also increased the efficiency of sample preparation. For animal tissues, elements more easily released were efficiently recovered without any addition of combustion aids. On the other hand, for most of the elements in the remaining samples, specially milk powder, the use of ethanol and paraffin, as well as a cooling step, were procedures necessary to increase combustion efficiency. Fig. 2 illustrates the improvement of element recoveries by addition of a second combustion aid (paraffin) and a cooling step of 5–10 min. The same behavior was observed for most of the elements in corn bran, which recoveries reached from 90 to 100%. The contents of Zn for all samples were in agreement with certified values, with and without using combustion aids and

a cooling step. For low Cu contents the results are shown as its detection limit (see in Table 2 as < DL). No interference in the elemental determination by ICP-OES was observed by the addition of the combustion aids, neither the blank values increased. Chromium, Fe, Mn, and Ni contamination from the stainless steel vessel and fuse wire was also investigated. However, no blank contamination was observed within the detection limits of the spectrometer.

Table 3 shows the results of iodine recoveries in spiked milk samples. This element was successfully determined by ICP-OES with axial view configuration of the plasma torch. Experiments with three different amounts of KI and a 10-min cooling step, reached iodine recoveries over 100% for lower concentrations of KI added. Certainly, the cooling step significantly improved iodine recovery for all amounts spiked. In addition, no combustion aid was necessary for efficient decomposition. Iodine determination by ICP-OES was not affected by P and S, which emission lines are close to that of iodine.

Atmospheric nitrogen, which is usually partially burned into nitrogen oxides entrapped in the decomposition vessel at the start of the oxygen filling operation, did not affect the subsequent analysis.

Table 3

Iodine recoveries from milk samples (500 mg) after spiking with different concentrations of KI (mg l^{-1}): A, 154; B, 75; C, 38

Procedure	Iodine recovery (%)		
	A	B	C
With no cooling step	57 ± 5	72 ± 10	82 ± 1
With a cooling step	75 ± 6	96 ± 8	105 ± 1

Mean values \pm S.D. ($n=3$).

4. Conclusions

The procedure proposed seems to be an efficient method for preparation of biological samples. It uses a conventional low cost equipment usually found in laboratories dealing with calorimetric measurements of a variety of materials such as plastics, food and feedstuff, natural products, chemical products, ashes, slag, mineral oil, coal, soils minerals and sludge. In addition, the procedure applies small amounts of reagents at low concentrations, reducing hazardous wastes usually produced during chemical analyses. The possibility of working with sample masses as high as 500 mg improves representativity and precision. Even though it appears to be a slow method, the single oxygen bomb takes approximately 10–15 min for

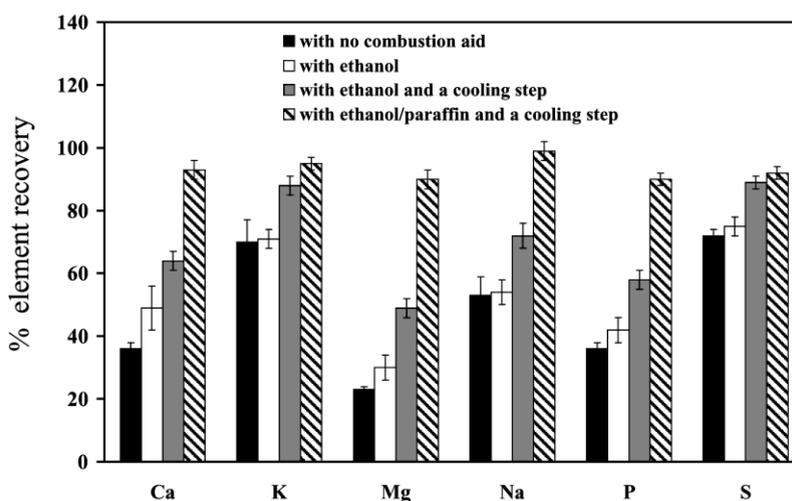


Fig. 2. Element recoveries from certified milk samples after variation in the decomposition parameters.

each sample preparation including all procedure steps.

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