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Improvement of a mineralisation method for fish flesh prior to analysis

Perfectionnement d'une méthode de minéralisation de chair de poisson préalablement à l'analyse

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ABSTRACT

Noureddine N., F. Nasfi, J. Zaouali - Improvement of a mineralisation method of fish flesh prior to analysis. Mar. Life, ***13*** *(1-2): 61-69.*

For routine analysis of total mercury content in foodstuffs, investigations were carried out in order to choose a suitable method of mineralisation of the organic matter, prior to analysis of its total mercury content. All known methods, starting from Klein's, were investigated. Dry mineralisation methods were discarded, due to the high degree of volatility of methyl mercury found in foodstuff. The wet mineralisation methods were tested included mineralisation in sealed funnel and by Vigreux refregiration with heating both by bunsen and by waterbath. Fish flesh was removed from three common fish species encountered year round on the Tunisian Coast. The sealed funnel mineralisation was discarded after successive breaking of funnels. The comparative Hg content after mineralisation of three different fish species, namely Mugil cephalus, Sarpa salpa *and* Mullus barbatus, *were recorded by flameless atomic absorption spectrophotometry. No statistically significant differences were found between the two Vigreux methods investigated. After finding major disadvantages in the use of reagents and catalysts, we developed our own method, presented in this report.*

RÉSUMÉ

Noureddine N., F. Nasfi, J. Zaouali - [Perfectionnement d'une méthode de minéralisation de chair de poisson préalablement à l'analyse]. Mar. Life, ***13*** *(1-2) : 61-69.*

Dans la perspective du dosage du mercure total dans des échantillons de poisson, nous nous sommes mis à la recherche d'une méthode de minéralisation fiable, simple et peu coûteuse. Pour cela, il nous a fallu passer en revue toutes les méthodes connues de minéralisation, partant de celle de Klein. Vue la grande volatilité du méthyl mercure, les méthodes de minéralisation par voie sèche ont été écartées. Nous nous sommes surtout intéressés à la minéralisation par voie humide. Nous avons ensuite testé trois méthodes de minéralisation par voie humide, en l'occurrence une minéralisation en ampoule hermétique et deux par réfrigération par colonne Vigreux, l'une chauffée par un bec Bunsen, l'autre en bain-marie. La chair de poisson utilisée a été retirée sur trois espèces de poisson présentes toute l'année sur les côtes de Tunisie, en l'occurrence : Mugil cephalus, Sarpa salpa *et* Mullus barbatus. *Les chairs des trois espèces de poisson minéralisées ont été soumises au dosage par spectrophotométrie d'absorption atomique. Ayant écarté la méthode de l'ampoule hermétique, la comparaison des deux autres n'a montré, statistiquement, aucune différence significative. Découvrant que des procédés, des réactifs et des catalyseurs présentaient de sérieux inconvénients, nous avons mis au point notre propre méthode de minéralisation que nous décrivons ici.*

INTRODUCTION

Prior to the analysis of total mercury in foodstuffs and organic matter, it is necessary to mineralise samples in order to release mercuric compounds. In foodstuffs, mercury is linked in bonds Hg-S (strong), Hg-NH or Hg-OH (weaker). A strong acid and an oxydifying reagent are needed to break these bonds. The organic mercury found in foodstuff, mainly methylmercury and phenylmercury, as well as mineral mercury is then turned into ions Hg^{2+}

$$\text{Organic Hg} \xrightarrow[\text{oxydifying}]{H^+} Hg^{2+}$$

If total mercury investigation is performed by flameless atomic absorption spectrophotometry, then, Hg2+ has to be reduced in Hg0 to be analysed by atomic absorption spectrophotometry, such as:

$$Hg^{2+} + 2\,e^- \longrightarrow Hg^0$$

Mercury Hg^0 is the only chemical shape detected by atomic absorption spectrophotometry.

Looking for a reliable mineralisation method, many known methods were reviewed and some of them were used, tested and compared, starting from Klein's (Klein, 1952) up to the most recent. These are summarized in tables I.1, and I.2.

With the knowledge that organo-mercurial compounds volatilize at temperatures below 100°C, methylmercury volatilizing at 70.5°C (Lide, 2000), we discarded nearly all the dry mineralisation methods.

Thus, only wet mineralisation methods remained. For the same raison, all non vapor-tight methods were discarded too.

This left only three methods, namely a dry mineralisation in a sealed funnel, and two wet methods involving heating over and one under 100°C, both cooled by a Vigreux cooler.

Once the choice of reagents had been made and sufficient quantities found, the duration and results of the three mineralisation methods chosen were analysed.

Mineralisation methods using catalysers and/or several reagents were discarded. Some catalysers and common reagents are not always mercury-free; thus, investigation of mercury in foodstuff needs to be done with mercury-free reagents which are much more expensive than the others (Nasfi, 1992).

MATERIAL AND METHODS

Samples

The foodstuff used for the various mineralisation methods was the flesh of common Tunisian fish species namely: *Mugil cephalus, Sarpa salpa, Mullus barbatus, Scomber scombrus, Sardina pilchardus, Thunnus thynnus* and *Scyliorhinus canicula,* all of them caught off the Tunisian shore.

Fish was washed and rinsed with demineralised water and the flesh removed and homogenised, from each species separately. Then, 4 g wet samples were taken for mineralisation.

Glassware washing

Glassware used was repeatedly and carefully washed, as follows.

Prewashing is done with a sulfo-chromic mixture. Rinsing is done with demineralised water. Washing with pure concentrated nitric acid of the "for toxicological analyses" type was then performed. A second rinsing is done with demineralised water. Drying is performed at 110°C in a drier.

Demineralised water was produced from a freshwater tap containing low average minerals at pH=7 and by a demineralising device, whose ions-exchange resin had been replaced prior to analysis. The demineralised water obtained was checked and proven to be mercury-free. This water was also used for dilution and water additions.

All chemical reagents were pure, of the "for toxicological analyses" type, to avoid mercury contamination by the reagents themselves. Contamination was avoided by using the least amount of glassware possible.

Glass funnels tightened by pincers and tight sets of erlenmeyers and Vigreux coolers were used.

4 g samples of wet fish flesh were mineralised in a glass funnel or in 150 mL erlenmeyers from the kit of a flameless atomic absorption spectrophotometer. The latter was a Perkin-Elmer type 420.

Using methods of dry and wet mineralisation of organic matter, it was found that the loss of mercury occured less when the sample underwent mineralisation under refrigeration or by the cooling method, unless mineralisation was in a sealed funnel.

This study investigated three mineralisation methods, one in a sealed funnel or dry mineralisation, and two methods of wet mineralisation cooled by Vigreux refrigeration, with different temperature values of the heated foodstuff:

- the first was heated by hot waterbath at 70°C;
- the second was heated to over 100°C with a Bunsen burner.

The samples underwent oxydation and acidification, during which the following chemical reagents were added:

- 5 mL HNO_3 oxydifying and acidifying
- 5 mL H_2SO_4 for the mineralisation of all Hg compounds

Immediately after addition of the second acid:

- the glass funnel was sealed with pincers and put in an oven heated to 80°C, for the first case,
- a 30 cm-long Vigreux cooler was placed over each erlenmeyer to avoid any loss of mercury due to temperature increase, in the two remaining cases. Tap water entering Vigreux cooler at 14°C left at ca.18°C.

Tables I(1) - Record of investigated methods of mineralisation, in chronological order. / Tableaux I(1) - *Méthodes de minéralisation retenues, dans un ordre chronologique.*

N°	Author	Issue year	Acids	Oxydifying reagents	Catalysor	Temp. °C	Duration hour	Needed Equipment	Cooling system T = Temperature °C = Celsius
1	Gorsuch	1970	HF	HNO_3	$KMnO_4$	<100	3	Waterbath + Reflux cooler	Room temperature
2	Wobeser *et al.*	1970	H_2SO_4	NH_4OCl		>100	2	Pressure coocker + sealed funnel	Ice
3	Uthe *et al.*	1970	H_2SO_4	$KMnO_4$		>100	3	Uthe's equipment + magnetic Shaker + burner	Room temperature
4	Uthe *et al.*	1971	H_2SO_4	$KMnO_4$		>100	3	Uthe's equipment + magnetic Shaker + burner	Room temperature
5	Lindstedt, Skäre	1971	H_2SO_4	$KMnO_4$		>100	3	Burner + Reflux cooler	Room temperature
6	Armstrong, Uthe	1971	H_2SO_4	HNO_3		58	2	Waterbath + Reflux cooler	Room temperature
7	Cumont	1971	H_2SO_4	$KMnO_4$		>100	3	Malaiyandi's equipment	Alcohol at - 75°C
8	Cumont	1971	H_2SO_4	HNO_3		150	2	Teflon sealed funnel + sandbath	-
9	Holak *et al.*	1972		HNO_3		150	1	Teflon sealed funnel in oven	Dry mineralisation
10	Nelson, Smith	1972	H_2SO_4	HNO_3		>100		Teflon sealed funnel in oven	Dry mineralisation
11	Cumont, Viallex	1972	H_2SO_4	HNO_3	$V_2O_5 + H_2O_2$		3	Malaiyandi's equipment	Alcohol at - 75°C
12	Carisano *et al.*	1972	H_2SO_4	$KMnO_4 + HNO_3$		>100	3	Burner + Reflux	Room temperature
13	Fukui *et al.*	1972	H_2SO_4	HNO_3		>100	3	Burner + Reflux	T° ambiante
14	Saha	1972	H_2SO_4	$KMnO_4$		>100	3	Burner + Reflux	Room temperature
15	Malaiyandi, Barette	1972	H_2SO_4	HNO_3	$V_2O_5 + H_2O_2$		3	Malaiyandi's equipment	Alcohol at - 75°C
16	Monteil	1972	H_2SO_4	$KMnO_4 + HNO_3$		>100	3	Burner + Reflux	Room temperature
17	Rains, Menis	1972	H_2SO_4	HNO_3		>100	3	Burner + Reflux	Room temperature
18	Rivers	1972		$KMnO_4 + HNO_3$		>100	3	Burner + Reflux	Room temperature
19	Lamn, Ruzicka	1972	H_2SO_4	$KMnO_4 + O_2$		>100	3	Oven	-
20	Cayrol, Brun	1973	H_2SO_4	$KMnO_4 + HNO_3$	NH_4OCl	58-60	6	Uthe's equipment	Alcohol at - 75°C
21	Cumont,Chevalier	1975	H_2SO_4	HNO_3		80	6-8	Sealed funnel + waterbath	Deepfreezing
22	Agemian	1976	H_2SO_4 + HCl	$KMnO_4 + HNO_3$ + $K_2S_2O_8$	NaCl + NH_2OH - H_2SO_4	20	3/4	Kjeldahl's bottle + magnetic shaker	-
23	Bacci *et al.*	1976	H_2SO_4	HNO_3 + NH2OH		>100	2	Friedrichs's equipment	Room temperature
24	Pearce *et al.*	1976		HNO_3		>100	2	Sealed funnel	Deepfreezing
25	Pearce *et al.*	1976		HNO_3		<100	-	Open tube in waterbath	-
26	Pearce *et al.*	1976	H_2SO_4	HNO_3		>100	-	Kjeldahl's bottler + Burner	-
27	A.M.C.	1977	H_2SO_4	$KMnO_4 + HNO_3$	$CuSO_4$	80	3	Kjeldahl's bottle	-
28	A.M.C.	1977	HCl H_2SO_4	$KMnO_4 + HNO_3$	$CuSO_4$+NaOH	80	3	Waterbath + Reflux	Room temperature
29	Kim *et al.*	1977	H_2SO_4	HNO_3	V_2O_5	>100	-	Burner + Reflux	Room temperature
30	Augier *et al.*	1978	H_2SO_4	HNO_3	V_2O_5	>100	3	Uthe's equipment	Alcool at - 75°C
31	Hattula *et al.*	1978	H_2SO_4	$KMnO_4 + NH_4OCl$		60	4	Waterbath + Reflux	Room temperature
32	Gardner *et al.*	1978	H_2SO_4	$KMnO_4 + HNO_3$	NH_2OH - H_2SO_4	60	3/4	Sealed funnel in wa terba th	-
33	Sorentino	1979	H_2SO_4	HNO_3		80	24	A.O.A.C. Apparatus	Icebath

Tables I(2) - Record of investigated methods of mineralisation, in chronological order. / Tableaux I(2) - *Méthodes de minéralisation retenues, dans un ordre chronologique.*

N°	Author	Issue year	Acids	Oxydifying reagents	Catalysor	Temp. °C	Duration hour	Needed Equipment	Cooling system T = Temperature °C = Celsius
34	Ramamurthy	1979	H_2SO_4	HNO_3		> 100	-	Burner + Reflux	Room temperature
35	Mondain, Gras	1980	H_2SO_4	$KMnO_4$		> 100	-	Burner + Reflux	Room temperature
36	Collett *et al.*	1980	HCl	KOH	$CuSO_4$	> 100	-	Markham's Apparatus	-
37	Luten *et al.*	1980		$HNO_3 + NH_4OCl$		>100	3	Teflon sealed funnel in oven	-
38	Gras, Mondain	1982	H_2SO_4	$HNO_3 + KMnO_4$		>100	-	Burner + Reflux	Room temperature
39	Dehairs *et al.*	1982	$HF + H_2SO_4$	$KMnO_4 + HNO_3$	NH_4OCl	110	5	Sealed funnel in waterbath	-
40	Dehairs *et al.*	1982	H_2SO_4	$KMnO_4 + HNO_3$	$NH_4O + H_2O_2$	60	-	Waterbath + Reflux	Ambient T
41	Cappon, Crispin Smith	1982 (a)	HCl	$HClO_4$	$Na_2Cr_2O_7$	>100	-	Burner + Reflux	Room temperature
42	Cappon, Crispin Smith	1982(b)	HCl	$HClO_4$ + Urea	$CuSO_4$	>100	-	Burner + Reflux	Room temperature
43	Shrivasta, Tandon	1982	H_2SO_4	$HNO_3 + KMnO_4$		>100	-	Burner + Reflux	Room temperature
44	Way	1983	H_2SO_4 + HCl	HNO_3		<100	1	Covered Erlenmeyer in waterbath	-
45	James	1983	H_2SO_4	$KMnO_4$		>100	3	Burner + Reflux cooler	Room temperature
46	Sadiq, Zaidi	1983	H_2SO_4	HNO_3		<100	4 - 6	Waterbath + Reflux	Room temperature
47	Vibhakar *et al.*	1983	HCl	HNO_3		>100	-	Burner + Reflux	Room temperature
48	Lodenius *et al.*	1983	H_2SO_4	HNO_3		60	4	Waterbath + Reflux	Room temperature
49	Breittmayer, Zsurger	1983	$HClO_4$	HNO_3		80	4	Waterbath + Reflux	Room temperature
50	Hight, Capar	1983	H_2SO_4	HNO_3		60	2-3	Waterbath + Reflux	Room temperature
51	Golimowski, Gustavsson	1984	$HClO_4$	HNO_3		>100	-	Burner + Reflux	Room temperature
52	Janssen, Brûne	1984	H_2SO_4	HNO_3		60	4	Waterbath + Reflux	Room temperature
53	Hardisson *et al.*	1985	H_2SO_4	HNO_3		40-50	11	Waterbath + Reflux	Room temperature
54	Pharmacopée	1987	H_2SO_4	HNO_3		>100	1	Burner + Reflux	Room temperature
55	Medina *et al.*	1988	H_2SO_4	HNO_3	$NaBH_4$	>100	1	Burner + Reflux Vector gas = Argon	Room temperature
56	Livardjani *et al.*	1991	H_2SO_4	HNO_3	$KMnO_4$ $Sn\ Cl_2$	>100	1	Burner + Reflux	Room temperature
57	Livardjani *et al.*	1994	H_2SO_4	HNO_3	$KMnO_4$ $(CO_2H)_2$	>100	1	Burner + Reflux	Room temperature
58	Puk, Weber	1994	H_2SO_4	HNO_3	$KMnO_4$	>100	1	Burner + Reflux	Room temperature
59	Livardjani *et al.*	1995	H_2SO_4	HNO_3	$KMnO_4$ $(CO_2H)_2$	>100	1	Electric Burner + Reflux	Room temperature
60	Noureddine *et al.* (à paraître)	2003	H_2SO_4	HNO_3		>100	1	Burner + Reflux	Room temperature

The erlenmeyers were then placed:
- in a waterbath at 70°C;
- over a Bunsen burner at over 100°C;

depending upon which of the two methods was used.

Duration of the mineralisation depended upon the temperature in each case. Mineralisation was completed when a sample turned to a clear aliquot. This state was reached after four hours for the waterbath method and after one hour for the Bunsen-heated method. When the heat was stopped, 15 mL demineralised water were added from the top of the "Vigreux" cooler to each erlenmeyer. This water washed the cooler walls in case some mercury was still suspended there. The mineralised and liquified samples were allowed to cool for two hours before reduction of mercury started.

The reduction of mercury took place by adding 25 mL $SnCl_2$ (pure, "for toxicological analyses, containing a maximum of 1.10^{-7}% Hg") turning Hg^{2+} into Hg^0 as shown by the above reaction.

($SnCl_2$, was prepared as follows: 20 g $SnCl_2$, in heated HCl, 3 N, qsp 100 mL).

All mercury has to be turned into Hg^0, because Hg^0 is the only chemical state under which mercury can be detected.

Immediately after $SnCl_2$ addition, each erlenmeyer was placed and sealed in the spectrophotometer kit, to avoid any loss of mercury. Then, detection and evaluation of mercury started by flameless atomic absorption spectrophotometry.

The reduced Hg^0 mercury (by $SnCl_2$), was vaporised and transported by a small circulation pump to a quartz cell. The cell was crossed by the

Table II - Increase of temperature during digestion consecutive to addition of an acid to flesh of fish. / *Augmentation de température consécutive à l'addition d'acide à la chair de poisson.*

Sample Fish Species	Addition of reagents on the samples	Rise of temperature in °C		
		Erlenmeyer on wet area	Erlenmeyer on dry area without shaking	Erlenmeyer on dry area with shaking
Mugil	+ H_2SO_4	16-19	26-30	31-36
Mullus	+ HNO_3	19-22	40-50	44-53
Sarpa et Sardina	+ H_2SO_4	27-33	37-45	39-47
Scomber	+ HNO_3	35-41	39-47	52-63
Scyliorhinus	+ H_2SO_4	23-30	29-36	35-43
Thunnus	+ HNO_3	31-40	41-47	50-61

Table III: Hg concentrations (mg/Kg) in fish through two different wet mineralisation methods. / *Tableau III : Concentrations en Hg (mg/Kg) dans la chair de poisson obtenues par deux méthodes de minéralisation.*

Fish species	*Mugil cephalus*		*Sarpa salpa*		*Mullus barbatus*	
Method	Burner > 100°C	Waterbath = 70°C	Burner > 100°C	Waterbath = 70°C	Burner > 100°C	Waterbath = 70°C
Minimum content in p.p.b	8.600	8.600	17.800	16.800	25.200	26.600
Maximum content in p.p.b	12.100	10.300	20.000	20.400	28.900	29.000
Mean in p.p.b	9.420	9.290	18.660	18.540	27.020	27.210
Std deviation	1.047	0.495	0.641	0.977	0.884	0.883
Paired observations	10	10	10	10	10	10

Table IV - Data means and standard deviation for burner and waterbath methods. / *Moyennes et écarts type relevés au cours des méthodes Bunsen et bain-marie.*

	Total Hg mean concentration (in µg.kg^{-1})		
Fish species	*Mugil cephalus*	*Sarpa salpa*	*Mullus barbatus*
Burner > 100°C	9.420	18.660	27.020
Waterbath = 70°C	9.290	18.540	27.210
Standard deviation	0.092	0.085	0.134

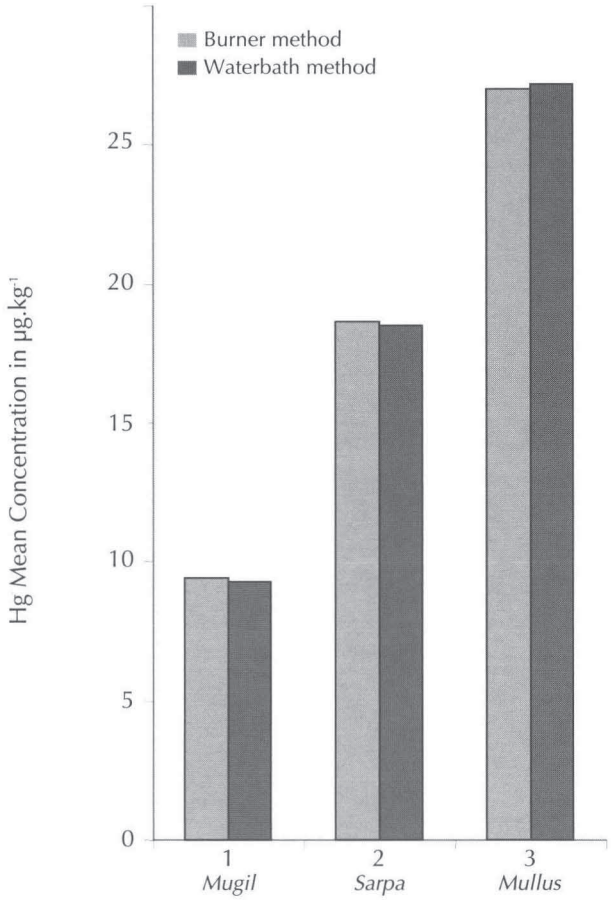

Figure 1 – Comparison of burner and waterbath mineralisation. / *Comparaison entre minéralisations "Bunsen" et "bain-marie".*

main mercury resonance ray, i.e. 253.7 nm. Apart from atomic fluorescence, flameless atomic absorption spectrophotometry provides the best assessments of total mercury: Thilliez, 1968; Thorpe, 1971; Munns, 1971; Boucetta *et al.*, 1972; Lamn, Ruzicka, 1972; Malaiyandi, Barette, 1972; Nelson, Smith, 1972; Hoggins, 1973; Analytical Methods Committee, 1977; Collett *et al.*, 1980; Breittmayer, Zsurger, 1983; Way, 1983; Janssen, Brûne, 1984; Hardinson *et al.*, 1985 and Pinstock, Umland, 1985.

In a by-side operation, oxydifying reagents as $KMnO_4$, oxalic acid, oxydifying catalysers likewise $CuSO_4$, V_2O_5, $Na_2Cr_2O_7$, Na_2MoO_4 and H_2O_2, were added to some samples.

Ten samples were taken for each fish species. Six evaluations were made for each sample. The results are given in tables III and IV.

RESULTS

The sealed funnels broke six times in an oven heated to 80°C, and even in a waterbath at 70°C. So the sealed funnel method was discarded, and solely wet mineralisation was left for consideration.

As far as wet mineralisation is concerned, the temperature of all samples was constantly checked, during digestion (maceration in acids). In no case was the volatilisation temperature of any organo-mercurial compound reached, i.e. 70.5°C (Lide, 2000) to 92.5°C (Carty, Malone, 1979).

The highest temperatures reached (63°C) were those obtained from mackerel samples, after addition of HNO_3 (table II).

The results obtained by the two "under-reflux" methods of mineralisation are given in table III which shows in p.p.b. the total Hg content (minimum concentration, maximum concentration and means) as well as the standard deviation and the number of paired observations for the different fish species, namely *Mugil cephalus, Sarpa salpa* and *Mullus barbatus.*

For all fish species and with both mineralisation methods, standard deviation range between 0.495 and 0.977, also less than 1 (1<), except for *Mugil cephalus* mineralised by the burner method, giving a standard deviation approximately equal to 1 (std deviation = 1.047), as it is shown in table III.

Standard deviation between burner and waterbath methods for the three fish species (table IV) ranges between 0.0848 and 0.134, and amounts to less than 0.1 for *Mugil* and *Sarpa.*

For both methods, results are as close as if they were provided by the same method. Although they derive from two different methods. This is well illustrated by figure 1 showing the two methods side by side.

No statistically significant differences were found between the two investigated methods, for any of the fish species (Anova, 1986).

So wet mineralisation "under-reflux" gives the same results, regardless of the type of organic matter (fish species in this case) and the temperature as long as it exceeds 60°C.

DISCUSSION

Heated mineralisations by Bunsen burner at temperatures over these of mercury volatilisation (70.5°C) gave no measurable loss of mercury. For a given sample, this method gave the same result as the waterbath method with samples heated to a lower temperature (table III).

The same result was reached by the two methods, although one lasted one hour and the other four hours.

Irrespective of temperature, both devices were cooled by a Vigreux cooler to avoid any mercury loss.

Adding catalysers, such as V_2O_5, or $Na_2Cr_2O_7$, gave no significant difference, no gain of time or any other advantage. On the other hand, their disadvantage is their high cost and their ability

to contaminate the samples as they may contain trace metal such as mercury.

Adding oxydifying agents or others, such as $KMnO_4$, gave no significant difference, but raised the mercury content of the sample, as $KMnO_4$ often contains mercury (Nasfi, 1995).

To avoid mercury contamination by the reagents themselves, the use of $KMnO_4$, oxalic acid, oxydifying catalysers likewise $CuSO_4$, V_2O_5, $Na_2Cr_2O_7$, Na_2MoO_4 or oxydifying reagents like H_2O_2, was not considered for the main experiments.

To prevent errors of analysis, at the authors' point of view, addition of such reagents and catalysers (which may contain trace amounts of mercury) should be avoided unless their use is imperative for the success of the mineralisation.

CONCLUSION

As no statistically significant differences were recorded in the results from the two wet methods of mineralisation, it may be concluded that the Bunsen burner method is to be preferred, because it takes one hour to perform, compared with four hours for the waterbath method.

For more convenience, the Bunsen burner could be replaced by a thermostatically controlled electric heater. This would heat at a constant rate, giving better mineralisation and easier use.

Finally, from the results of the present experiments, it can be stated that wet mineralisation of a 4 g wet sample or 1 g dry sample should be done by 5 mL HNO_3 + 5 mL H_2SO_4 (pure, for toxicological analysis) solely.

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