ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 463–473

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Determination of mineral content of active dry yeast used in pharmaceutical formulations

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Received 3 August 2001; received in revised form 4 September 2001; accepted 30 September 2001

Abstract

The efficiency of seven common methods of digestion of active dry yeast (ADY), which is used in anticariogenic dental formulations, was evaluated for the analytical determination of Fe, Zn, Ca, Mg, Na and K. Four wet-acid digestion and three dry ashing methods are compared in consideration of their estimated reproducibility and metal concentrations obtained. HNO_3, $HNO_3 + HCl$, $HNO_3 + H_2SO_4$ and $HNO_3 + HClO_4$ were applied for wet digestion of the samples in medium temperatures, while dry ashing at higher temperature with $Mg(NO_3)_2$ or $SrCl_2$ as ashing aid agents, were the alternative methods. The final solutions were subsequently analyzed for Fe, Zn, Ca and Mg by flame atomic absorption spectroscopy (FAAS) and for Na and K by atomic emission spectroscopy (AES). Two multivariate statistical methodologies, Analysis of variance (ANOVA) and the Kruskal–Wallis test were applied for the interpretation of the results. Seven additional statistical tests (least-significant difference, Bonferoni, Duncan multiple range, Student–Newman–Keuls, Tuckey significant difference, Tuckey b and Scheffe) were used and proved useful to estimate which of the decomposition methods are outliers. The ideographic approach enabled the comparison of the methods in terms of complexity and difficulty of their steps. Zn and Mg could be reliably determined by any one of the tested methods, while for the other elements, the most powerful method was found and the obtained recoveries were found ($> 95\%$).

Keywords: Yeast; Minerals; Decomposition methods; Atomic spectroscopy; Pharmaceuticals

1. Introduction

Yeast is grown from ascomycetes *Saccharomyces cerevisiae* in large ferments by the fed batch process (FBP) [1]. The ferments contain cane or beet molasses, which supply not only sugars but also some organic nitrogen, sulfur, vitamins and inorganic elements. The liquid growth medium must be supplemented with additional nitrogen and phosphorus, vitamins, minerals (Ca, Mg), and other inorganic elements. During the post-fermentation process a concentrate yeast cream is formed and the active dry yeast (ADY) is finally produced by extrusion and

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00601-X

air-drying. The resulted product is consisted of high organic content.

The role of yeast in caries protection is well known [2]. It has an anticariogenic effect on enamel and offers considerable protection from caries. This protection was much more effective if fresh (not baked) yeast is used. Also, the mineral content (especially calcium) plays an important role preventing the decalcification process. For this reason pharmaceutical formulations, such as K-DENT® from Cosmopharm Ltd., were appeared in the market. Each capsule of the above formulations contains 100 mg yeast, 300 mg xylitole, 100 mg manitole and 1% m/m natural orange. Thus, the determination of various inorganic elements in yeast matrices is useful in pharmaceutical industry. Additionally, the determination of such elements in yeast matrices is useful for biological [3], nutritional and toxicological studies [4].

The most common approach to analyze such matrices is the application of effective and reproducible decomposition techniques for sample treatment. Generally, decomposition of carbon-rich samples is a more tedious work than that of carbon-poor samples because breaking down of carbon matrix requires both higher temperatures and strong oxidative acid mixtures [5]. The use of mineral acids either alone [6–9] or in mixtures [10–12] is the most common approach for wet digestion of yeast samples. Concentrated acids such as HNO_3, H_2SO_4, HCl, $HClO_4$, as well as double or triple mixtures of them, with or without other oxidants have also been used. In parallel, many efficient dry ashing methods have been developed and applied to similar matrices [5,13].

Also, the use of multivariate statistical methods enables the estimation of similarities in the efficiency of decomposition methods for the same or different analytes, and becomes a useful tool in critical comparison of a number of such decomposition methods [13,14].

In literature, there is a lack of analytical procedures concerning the mineral composition analysis of baker's yeast matrix and, furthermore, to the best of our knowledge, no comparative use of decomposition methods has been reported for yeast. So the purpose of this work was the comparative investigation of four wet digestion and three dry ashing methods for determination of the most common metals in baker's ADY used in production of dental formulations, which are Fe, Zn, Ca, Mg, Na and K [15], by atomic absorption and atomic emission spectroscopy (AES).

2. Materials and methods

2.1. Instrumentation and reagents

All chemicals were of analytical grade and were provided by Merck (Darmstadt, Germany) unless stated otherwise, and all the solutions were made up in de-ionized water. Concentrated hydrochloric (1.19 g ml^{-1}), nitric (1.40 g ml^{-1}), sulphuric (1.84 g ml^{-1}) and perchloric acid (1.67 g ml^{-1}), were used for the wet digestion. Before the dry ashing, 0.3 g $Mg(NO_3)_2 \cdot 6H_2O$ (3 ml of 10% m V^{-1} alcoholic solution) or 0.2 g $SrCl_2$ (2 ml of 10% m V^{-1} aqueous solution) were added in the samples, as ashing aid agents, in two of the three dry ashing procedures investigated.

The standard stock solutions of Fe, Zn, Ca, Mg, Na, and K 1000 mg l^{-1} were prepared in 0.5 M HNO_3 and the working solutions of metals were prepared by stepwise dilution immediately before use. As far as we know, no certified reference yeast material was available for metals, so a bulk of commercial ADY was used for the comparative investigations of different methods. For want of certificate of the sample, the efficiency of methods was tested by means of the higher metal concentration obtained, and the precision was calculated by repeated analyses.

The determination of Fe, Zn, Ca and Mg was performed by a Perkin–Elmer (Uberlingen) Model 5100 Atomic Absorption Spectrophotometer. Na and K were determined by a Lange (Berlin) M6a Atomic Emission Flame Spectrophotometer. A sand-bath was used for wet digestion, while a muffle furnace was used for dry ashing. In all procedures, open decomposition vessels (pyrex glass beakers, conical flasks or porcelain crucibles) were used.

2.2. Decomposition methods

Seven digestion procedures, which are used in routine analysis, were investigated. The wet-acid digestion methods are symbolized by letter W while the dry ashing ones by letter D. Five subsamples of 1 g of commercial ADY for each individual method are treated as follows.

2.2.1. Method W 1

The sample is treated with 10 ml of concentrated HNO_3 in a 100 ml conical flask and is heated gradually to 350 °C, until almost dryness. Then the residue is diluted to 100 ml with double de-ionized water [9,16]. Decompositions with only nitric acid are usually less efficient for highly organic than for inorganic matter, and in case of yeast samples the heating to dryness should be performed more times.

2.2.2. Method W 2

The sample is treated with 10 ml of concentrated HNO_3 in a 100 ml conical flask (similar volume kjeldahl flasks can be used also), then heated gradually to 400 °C until almost dryness, and is left to cool. The residue is dissolved in 10 ml of (1 + 1) HCl and is heated to 200 °C until almost dryness. Then it is diluted to 100 ml with double de-ionized water. Although the use of these acids is preferable for inorganic samples, metals, alloys, etc., it was tested also for yeast, because HCl is less difficult to handle than H_2SO_4 and $HClO_4$.

2.2.3. Method W 3

The sample is treated with 10 ml of concentrated HNO_3 in a 100 ml conical flask (similar volume kjeldahl flasks can be used also), then is heated gradually to 350 °C, until evolution of nitrogen oxides ceases, and is left to cool. The residue is dissolved in 3 ml of concentrated H_2SO_4 and is heated again to 350 °C until white fumes of sulphur oxides. Then it is diluted to 100 ml with double de-ionized water [10,16]. It should be mentioned here, that the order of the additions of nitric and sulphuric acids could be reversed, with similar efficiency. On the other hand, the simultaneous use of the two acids in mixture was not equally efficient, and was not tested further.

2.2.4. Method W 4

The sample is treated with 10 ml of concentrated HNO_3 in a 100 ml conical flask (similar volume kjeldahl flasks can be used also), then is heated gradually to 250 °C until almost dryness, and it is left to cool. The residue is dissolved in 2 ml concentrated $HClO_4$ and is heated again to 250 °C until fumes of perchloric acid. In some cases, more additions of perchloric acid may be necessary to obtain clear solutions. Then, it is diluted to 100 ml with double de-ionized water [5,11]. Care should be taken to preliminary oxidize the bulk organic matter with HNO_3, in order to avoid explosion when $HClO_4$ is added.

2.2.5. Method D1

The sample is placed in a porcelain crucible and is ashed at 550 °C for 8–12 h. Then it is dissolved in 5 ml of 0.6 mol l^{-1} HNO_3 and the solution is diluted to 100 ml with double de-ionized water. The temperature of 550 °C was selected as optimum, because at temperatures less than 450 °C a lot of organic substances are not burned off, while at temperatures more than 600 °C, some inorganic salts of the metals can be lost.

2.2.6. Method D2

The sample is placed in a porcelain crucible with 0.3 g of $Mg(NO_3)_2$ (3 ml of 10% m V^{-1} alcoholic solution) and is ashed at 550 °C for 8–12 h. Then it is dissolved in 5 ml of 0.6 mol l^{-1} HNO_3 and the solution is diluted to 100 ml with double de-ionized water [9,16]. When using $Mg(NO_3)_2$ the active substance is the nitrate anion for its oxidizing properties.

2.2.7. Method D3

The sample is placed in a porcelain crucible with 0.2 g of $SrCl_2$ (2 ml of 10% m V^{-1} aqueous solution) and is ashed at 550 °C for 8–12 h. Then, it is dissolved in 5 ml of 0.6 mol l^{-1} HNO_3 and the solution is diluted to 100 ml with double de-ionized water. Ashing aids like $SrCl_2$ and other alkaline earth metals serve in that the volume of the produced ash is much larger than without these agents, and the analytes are present at greater dilution in the ash and thus in less contact with the crucible surface.

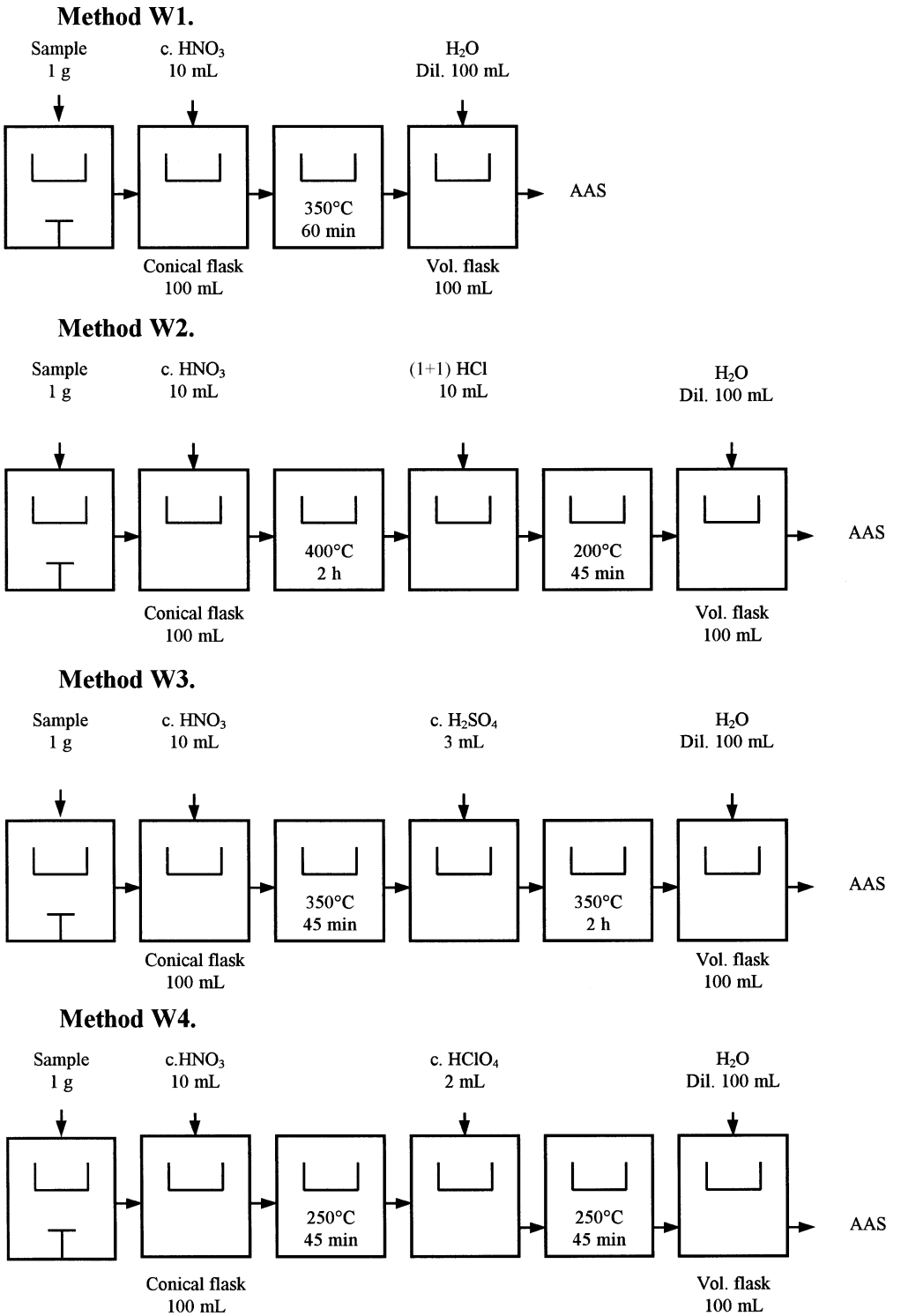
Method W1.
Sample
1 g
c. HNO3
10 mL
H2O
Dil. 100 mL
350°C
60 min
AAS
Conical flask
100 mL
Vol. flask
100 mL
Method W2.
Sample
1 g
c. HNO3
10 mL
(1+1) HCl
10 mL
H2O
Dil. 100 mL
400°C
2 h
200°C
45 min
AAS
Conical flask
100 mL
Vol. flask
100 mL
Method W3.
Sample
1 g
c. HNO3
10 mL
c. H2SO4
3 mL
H2O
Dil. 100 mL
350°C
45 min
350°C
2 h
AAS
Conical flask
100 mL
Vol. flask
100 mL
Method W4.
Sample
1 g
c.HNO3
10 mL
c. HClO4
2 mL
H2O
Dil. 100 mL
250°C
45 min
250°C
45 min
AAS
Conical flask
100 mL
Vol. flask
100 mL

Fig. 1.

In yeast samples, carbon is the predominant matrix element. Depending on the determination method, the decomposition technique must give a homogeneous and clear solution in which, more or less, carbon residues are tolerable. The dry ashing methods met the expectations for homogeneous, particle-free, transparent, and almost colorless solution, while, a slight turbidity was remained in the solutions obtained after wet digestion methods. A centrifuging step was needed, in case of methods W1 and W2. The prepared solutions according to the above methods are injected directly into the instrument or after suitable dilution with double de-ionized water. All the analytes are minor constituents at concentrations > 10 mg kg^{-1} and such levels are easily detectable by flame atomic spectroscopy, which has lower detection limits and sufficient sensitivity.

3. Results and discussion

In Figs. 1 and 2, the ideographic comparison of the four wet digestion methods and the three dry ashing methods is illustrated. This approach was made according to Malissa and Simeonov [17] and Ortner and Scherer [18]. This schematic description is very useful for a rapid evaluation of the complexity of a method, especially in cases where many methods must be compared simultaneously.

It is obvious that method W1 includes fewer steps than D1–D3 and even fewer than W2–W4. So, judging from the time-period, methods W1 and W4 are faster, followed by method W2 and all the others. It should be mentioned that care should be taken to purify the reagents and water, when wet acid digestion methods are applied for sample decomposition, since large amounts of acid reagents are used. On the other hand, when dry ashing methods D1–D3 are applied, we must also take into consideration that they are time-consuming, since the crucible has to be heated extensively in the furnace. In addition, the ashing temperature must be carefully controlled or else the volatile elements would be lost.

The reagents and apparatus used in all digestions are common and cheap, and the seven methods are comparable in the sense that they make use of equal quantities of sample. All the tested methods involve open vessels. Open-vessel methods have the advantages of quick reagent additions and easy attendance of the mixture condition over closed-vessel methods, although the last ones have the advantage of negligible analyte losses.

Mean concentrations $\pm$ standard deviations (S.D.) are listed in Table 1. According to method D2, magnesium could not be determined because of the addition of $Mg(NO_3)_2$, as an ashing aid agent. Statistical analysis attempts to support null hypothesis, H_0 or adopt its alternative one, H_1. Thus, in this case, the null hypothesis is considered to be H_0, none of the 7 digestion methods differs significantly from the others. Consequently, the alternative H_1, one or more of the 7 digestion methods differ significantly from the others (showing higher or lower values).

In case that the results are considered as parametrical (i.e. s ratio follows the F distribution), a one-way analysis of variance (ANOVA) test is performed [19]. In fact, the data are probably not normally distributed, and the standard deviations for some of the elements show large differences. Under these circumstances, the use of Kruskal–Wallis test is better recommended. The groups of values to be compared are 7, as there is a separate result series for each individual method. These values are not provided by the application of all the methods on the same sample, but by the application of each one on a different sample (sub-sample). Statistical software (SPSS™, version 6.1) was used for the statistical treatment of the data.

The results of the application of ANOVA test are given in Table 2 and those of the application of Kruskal–Wallis test are given in Table 3. Based on these tables, the following are concluded, for probability level 95% ($P = 0.05$).

Fig. 1. Ideographic description of the four wet digestion procedures tested for ADY samples.

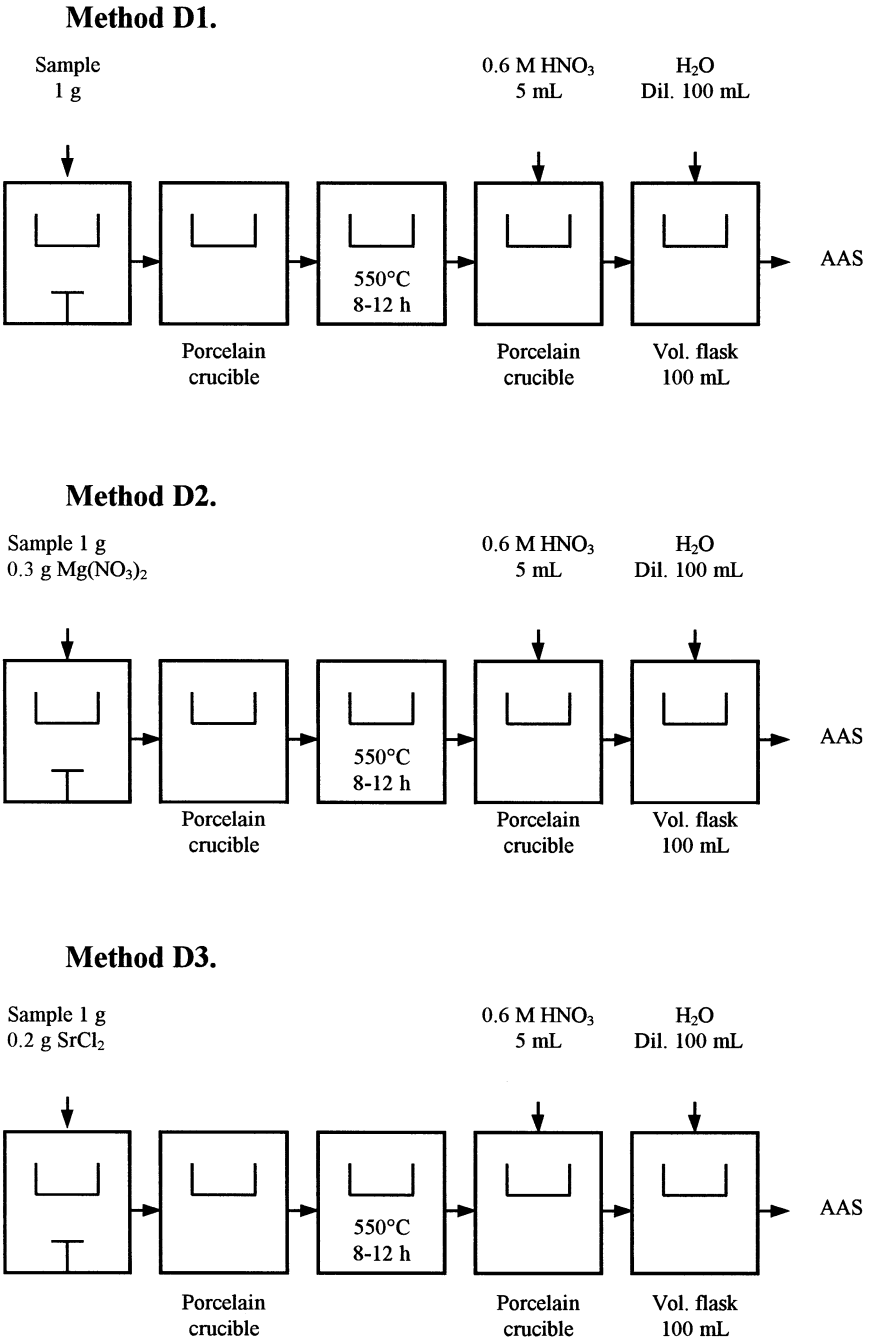

Fig. 2. Ideographic description of the three dry ashing procedures tested for ADY samples.

3.1. Iron

Both tests give statistically significant results ($P = 0.007$ and $P = 0.021$, respectively). Consequently, one or more of the performed decomposition methods differs significantly from the others. The application of more specific tests (see below) is required for the determination of the

difference. Methods W3 and D3 give higher results than all the other methods in most additional tests, and also their precision was comparable. The reason for the higher values obtained by method D3 is probably the presence of large amount of $SrCl_2$ which act as an ionization buffer during the atomization in atomic absorption spectrometry. The recovery of 50 mg kg^{-1} Fe spiked to eight sub-samples of yeast ranged between $98.0 \pm 4.7\%$.

3.2. Zinc

Both tests do not give statistically significant results ($P = 0.1092$ and $P = 0.1283$, respectively). Thus, none of the performed methods differ significantly from the others, although method D3 seems to slightly differ leading to higher values, but with insufficient precision. Application of method D1 to eight sub-samples of yeast spiked with 20 mg kg^{-1} Zn gave a recovery range between $96.7 \pm 3.4\%$.

3.3. Calcium

Both tests produce statistically very significant results ($P = 0.001$ and $P = 0.005$, respectively). So, one or more of the performed methods differs significantly from the others. Nevertheless, it is almost sure that method W2 gives lower results than all the other decomposition methods, and this is highlighted with the additional statistical tests (see below). The group of dry ashing methods shows greater variation than the group of wet digestion methods, and also method D1, gave higher mean value probably due to the existence of one outlier, which could not be rejected after application of Dixon's Q-test. Thus, methods W1 or W4 are the most efficient, and application of these two methods to eight sub-samples of yeast spiked with 200 mg kg^{-1} of Ca gave a recovery of $97.2 \pm 3.6\%$.

3.4. Magnesium

Both tests do not give statistically significant results ($P = 0.273$ and $P = 0.250$, respectively). Consequently, none of the performed methods differs significantly from the others. Moreover, it should be mentioned, that all methods (except D2 which makes use of $Mg(NO_3)_2$) produce comparable mean values, but methods W1 or D3 are the most efficient. It can be seen that the use of HNO_3, in method W1, is sufficient for magnesium recovery. Additionaly, the application of method D3 is slightly advantageous due to the introduction of $SrCl_2$, which enhance the atomization of magnesium. To eight samples spiked with 400 mg kg^{-1} of Mg a recovery of $98.2 \pm 2.5\%$ was obtained.

Table 1
Concentration of inorganic elements in analyzed ADY

Method (mg kg^{-1}) [a]	Iron (mg kg^{-1}) [b]	Zinc (mg kg^{-1})	Calcium (mg kg^{-1})	Magnesium (mg kg^{-1})	Sodium (mg kg^{-1})	Potassium (g kg^{-1})
W1	134.1 ± 10.6	52.9 ± 1.2	391.1 ± 8.7	883.5 ± 22.4	375.4 ± 11.8	15.00 ± 0.29
W2	134.1 ± 4.9	53.2 ± 1.4	226.9 ± 39.5	867.1 ± 16.8	576.3 ± 29.0	14.47 ± 0.44
W3	150.6 ± 7.9	51.8 ± 1.0	343.5 ± 16.0	820.5 ± 83.1	354.2 ± 14.5	13.06 ± 0.16
W4	138.8 ± 3.2	56.6 ± 4.7	399.8 ± 5.3	888.5 ± 63.8	354.2 ± 14.5	14.41 ± 0.21
D1	133.3 ± 9.0	55.7 ± 1.8	439.7 ± 85.2	877.5 ± 15.6	405.3 ± 40.4	14.31 ± 0.34
D2	137.3 ± 3.4	53.2 ± 2.8	392.7 ± 32.3		387.7 ± 15.2	14.22 ± 0.34
D3	147.1 ± 5.9	67.3 ± 21.3	396.0 ± 62.0	885.0 ± 4.3	519.9 ± 122.1	14.41 ± 0.29

[a] See text for method description.
[b] Mean $\pm$ S.D.

Table 2
ANOVA results and interpretation

Analyte	*F*-ratio	Probability	Significant difference	Higher results	Lower results
Fe	4.077	0.007	Yes	W3, D3	–
Zn	1.999	0.109	No	–	–
Ca	5.491	0.001	Yes	–	W2
Mg	1.404	0.273	No	–	–
Na	20.102	0.000	Yes	W2, D3	–
K	18.994	0.000	Yes	W1	W3

3.5. Sodium

Both tests give statistically very significant results ($P = 0.000$ and $P = 0.001$, respectively). Consequently, one or more of the performed methods differs significantly from the others. Method W2 gives statistically higher results than the other decomposition methods, proving thus, that the sequential use of nitric and hydrochloric acids is preferable than the sequential use of nitric and sulphuric acids or nitric and perchloric acids, respectively. It is probable that the SO_4^{2-} or ClO_4^- anions remaining in the final solution, which is injected to the nebulizer, cause a negative interference on the atomization process. Method D3 is also shown to give higher results according to many other additional tests (see below). This occurs for the same reason as it was described above for Fe and Mg. However, the performance of this method should be judged in relation with the higher standard deviation observed. Thus, the recovery range obtained by application of method W2 to eight sub-samples of yeast spiked with 200 mg kg^{-1} Na was $96.0 \pm 4.3\%$.

3.6. Potassium

Both tests give statistically very significant results ($P = 0.000$ and $P = 0.004$, respectively). Thus, one or more of the performed methods differs significantly from the others. Method W3 leads to constantly lower results than all the others, while method W1 gives higher results in most additional tests. A reasonable explanation for this observation, is that potassium is recovered efficiently even with nitric acid alone, and its atomization is probably affected by the presence of the SO_4^{2-} anions introduced with method W3. In addition, for method W4, which involves the reaction with perchloric acid, although it was expected to give lower results, those were not observed. On the other hand, the group of dry ashing methods shows equal efficiency and good reproducibility. Finally, application of method W1 to eight subsamples spiked with 2 g kg^{-1} of K gave a recovery range of $98.4 \pm 2.2\%$

3.7. Additional statistical tests

Although the above two tests are capable of tracing whether one or more of the performed methods differs significantly from the others, they are incapable of determining exactly which ones show the highest or lowest results. In literature, a series of typical tests managing to fulfill this task is referred [20,21]. These additional statistical tests are characterized as 'multiple comparisons' and the most powerful (with the abbreviations used below) are the least-significant difference (LSD), Bonferroni test (BON), Duncan multiple range test (DMR), Student–Newman–Keuls test (SNK), Tuckey honestly-significant difference (HSD), Tuckey b (TUC) and Scheffe (SCH). The LSD test, proposed by Fischer, is probably the most simple approach for comparing the absolute difference of a mean against several others. It is based on sequential paired comparisons, but with the suitable software this is not a laborious procedure. On the other hand, BON test uses the *t*-criterion for such comparisons, and the other tests are also rigorous approaches, based on a minimum distance criterion. This criterion is usually a statistical parameter calculated on the basis of the estimated pooled variance of the raw data.

The SCH method uses the criterion of 'linear contrast' which is an estimator based on the theoretical and sample mean values. Commonly, it is not applied to sequential paired comparisons. All the above mentioned tests are reliable but not equally sensitive, and the selection of the most appropriate one depends on the researcher's estimation.

For the needs of this study all seven tests were performed, using the SPSS™ statistical software. The results of the statistical analysis are presented in Table 4, where the efficiency of the statistical tests is compared. For some analytes all the tests were proved equally powerful and capable to reach the same conclusion, e.g. for Na method W2 gives undoubtedly the highest results, and the opposite is true for K and method W3. On the other hand, in case of Zn and Fe, it is worth to mention that the LSD and DMR were proved to be more sensitive, detecting some significant differences of D3 dry ashing method in comparison to most of the other decomposition methods. The other five tests failed to detect the differences for these two analytes. Finally, Scheffe test (SCH) did not detect the small differences occurring for Fe determination with method W3 and K determination with method W1.

4. Conclusions

It is proved that, for difficult matrices like yeast, insufficient recovery and bad reproducibility of the results for some elements are mostly attributed to unfit decomposition methods. For Zn and Mg determination, no statistically significant variation was observed among all the performed digestion procedures, so these metals could be determined reliably by any one of the tested methods. Using the dry ashing method in presence of $SrCl_2$, or wet digestion with the mixture of $HNO_3 + HCl$ significantly higher results were obtained for Na, and the same situation was observed for K using wet digestion with HNO_3. Significantly lower results for K were obtained by wet digestion with the mixture of $HNO_3 + H_2SO_4$ and for Ca with the mixture of $HNO_3 + HCl$, thus this procedures should be avoided in case of K and Ca determination, respectively. Good recoveries ($> 95\%$) are gained by application of the best procedures to spiked samples.

ANOVA and Kruskal–Wallis tests are useful tools for comparative chemometric purposes, while LSD and DMR were more sensitive for further detection of small differences. In the applications described above, both ANOVA and Kruskal–Wallis tests gave almost the same results, showing that the analytical results could be treated either as parametric or as non-parametric data. Finally, the ideographic approach was performed for the comparison of the digestion methods in terms of the complexity and difficulty of their steps. It was shown that wet digestion methods are faster for mineral composition analysis of yeast, but with more steps, while, dry ashing procedures are more simple but time-consuming.

Acknowledgements

The authors thank Dr V. Tachos, Assistant Professor of Preventive and Periodontological Dentistry, School of Dentistry, University of Thessaloniki.

Table 3
Kruskall–Wallis test results and interpretation

Analyte	χ^2-Factor	Probability	Significant difference	Higher results	Lower results
Fe	14.917	0.021	Yes	W3, D3	–
Zn	9.915	0.128	No	–	–
Ca	18.409	0.005	Yes	–	W2
Mg	5.393	0.250	No	–	–
Na	23.137	0.001	Yes	W2, D3	–
K	19.218	0.001	Yes	W1	W3

Table 4
Additional statistical tests for detection of higher and lower outliers

Analyte	Method [a]	Statistical test [b]						
		LSD	BON	DMR	SNK	HSD	TUC	SCH
Fe	W1							
	W2							
	W3	»	>	»	>	>	>	
	W4							
	D1							
	D2							
	D3	>		>				
Zn	W1							
	W2							
	W3							
	W4							
	D1							
	D2							
	D3	>		>				
Ca	W1							
	W2	«	<	«	«	<	«	<
	W3							
	W4							
	D1							
	D2							
	D3							
Mg	W1							
	W2							
	W3							
	W4							
	D1							
	D2							
	D3							
Na	W1							
	W2	»	»	»	»	»	»	»
	W3							
	W4							
	D1							
	D2							
	D3	»	>	»	»	»	»	>
K	W1	»	>	»	>	>	>	
	W2							
	W3	«	«	«	«	«	«	«
	W4							
	D1							
	D2							
	D3							

No sign, not significantly different results; » (or «), decomposition method gives higher (or lower) results than all the other methods; > (or <), decomposition method gives higher (or lower) results than most of the other methods.

[a] See text for method description.

[b] See text for test's abbreviations.

References

[1] G. Reed, T.W. Nagodawithana, Yeast Technology, second ed., Van Nostrand Reinhold, CT, 1990, pp. 261–314.
[2] V. Tachos, Second Congress of the European Academy of Pediatric Dentistry, June 18–21, Athens, Greece, 1994.
[3] D. Brady, J.R. Duncan, Enzyme Microbial. Tech. 16 (1994) 633–638.
[4] C. Askwith, J. Kaplan, Trends Biochem. Sci. 23 (1998) 135–138.
[5] Y. Ming, L. Bing, Spectrochim. Acta B 53 (1998) 1447–1454.
[6] L. Cuadros Rodriguez, C. Jimenez Linares, M. Roman-Ceba, Anal. Lett. 27 (1994) 1569–1577.
[7] M. Czauderna, E. Makowska, S. Smolinski, J. Radioanal. Nucl. Chem. 177 (1994) 393–401.
[8] L. Dunemann, M. Meinerling, Fresenius J. Anal. Chem. 342 (1992) 714–718.
[9] D.C. Reamer, C. Veillon, Anal. Chem. 53 (1981) 1192–1195.
[10] M.N. Arafat, W.A. Glooschenko, Analyst 106 (1981) 1174–1178.
[11] E.E. Cary, M. Rutzke, J. Assoc. Off. Anal. Chem. 66 (1983) 850–852.
[12] R. Saraswati, R.L. Watters, Talanta 41 (1994) 1785–1790.
[13] G. Zachariadis, J. Stratis, I. Kaniou, G. Kalligas, Mikrochim. Acta 119 (1995) 191–198.
[14] J. Stratis, G. Zachariadis, E. Dimitrakoudi, V. Simeonov, Fresenius J. Anal. Chem. 331 (1988) 725–729.
[15] B. Holland, A.A. Welch, I.D. Unwin, D.H. Buss, A.A. Paul, D.A.T. Southgate, McCance and Widdowson's: The Composition of Foods, fifth ed., Royal Society of Chemistry, Cambridge, 1998.
[16] R. Bock, A Handbook of Decomposition Methods in Analytical Chemistry, International Textbook Company, Edinburg, 1979, pp. 195–231 translated by Iain Marr.
[17] H. Malissa, V. Simeonov, Fresenius J. Anal. Chem. 289 (1978) 257–263.
[18] M.H. Ortner, A. Scherer, Talanta 24 (1977) 215–224.
[19] D. Massart, B. Vandeginste, L.C. Buydens, S. De Jong, P. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics A, Elsevier, Amsterdam, 1997, pp. 121–150.
[20] L. Stahle, S. Wold, Chemom. Intell. Lab. Syst. 9 (1990) 127–141.
[21] C. Chase, Elementary Statistical Procedures, third ed., McGraw Hill, New York, 1981, pp. 287–298.