RESEARCH ARTICLE

Optimization of digestion methods for mineral in spirullina by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)

R. Paranthaman, R.Vidyalakshmi, S.Kumaravel

Department of Food Safety & Quality Testing
Indian Institute of Crop Processing Technology, Ministry of Food Processing Industries
Thanjavur- 613 005, Tamil Nadu, India

J. Adv. Pharm. Edu. & Res.

ABSTRACT

Determination of elements from the spirullina powder by inductively coupled plasma-optical emission spectrometry (ICP-OES) and to report efficient, reliable, receptive method of microwave digestion for better recovery of the elements. The three digestion methods are studied included, dry ashing method and microwave digestion. ICP-OES The method has been validated using RF power of 1450 watts, plasma flow of 15L/min, Nebulizer flow of 0.7 L/min and plasma view at radial mode for all elements. As, Hg, Se, Zn, P, Pb, Cd, Fe, Mn, Cr, Mg, Cu, Ca, Na and K). The results of the elemental quantification were analyzed and the microwave digestion method shows exhibits good quantity of minerals like Mg, Ca , P, K and Fe. The microwave digestion method is found to more reliable as compare to wet digestion & dry ashing method and hence recommended for better recovery. The element Potassium (K),Magnesium (Mg), Phosphorous (P), Iron (Fe),Calcium (Ca) gives better recovery by ash and microwave digestion method as compare to wet digestion method. All the elements are quantified successfully by ICPOES technique by all methods of digestion. Dry and wet digestion methods are tedious and time consuming compare to microwave digestion method, which is greener and simpler. The spirullina popwder can be used as supplementary food for deficiency of minerals like Fe, Mg and Ca.

Keywords: spirullina powder, ICP-OES, Minerals, food quality, Microwave digestion

1. INTRODUCTION

Blue-green algae have been attracting the most attention as potential, indeed actual, sources of proteins and other human and natural nutrients (fatty acids, macro- and trace minerals). Among these algae, the filamentous cyanobacterium *Spirulina* appears to be a ubiquitous component of the phytoplankton growing in ocean and seawaters (from tropical lagoons to Arctic waters, thermal springs, alcaline lakes etc.) [1–3]. Further, the presence of an anti-oxidizing agent as β- carotene and the rare linolenic acids [4-6] suggests a beneficial therapeutic effect (first of all anti-cancer) of this algae. Spirulina is a nutrient rich super food for super health. Super foods can be defined as foods that have health promoting benefits and disease preventing properties over and above their usual nutritional value. Spirulina is the common name for human and animal food supplements produced primarily from two species of Cynobacteria, that is, *Arthrospireaplatensis* and *Arthrospira maxima*. Spirulina has many therapeuticproperties such as hypocholestrolemic, immunological, antiviral and antiglutagenic effects [7].Spirulina is an excellent source of protein. Today, food is lower in essential nutrients than foods produced 50 years ago. Farming practices have depleted our soil fertility (http://www.spirulinasource.com). Stress from environmental pollutants and lifestyle demands have increased our dietary requirements for certain essential nutrients. To overcome these problems, some super foods like aloe vera, garlic, tomato, walnut, blue green algae (spirulina) have been introduced. Spirulina, Blue green algae (fusiformis) is being used as nutrient dense food materials in natural and health foods. It also has some potent nutrients and probiotic compounds that enhance health condition [8]. The objective of the present study was the analysis of trace element Potassium (K) , Magnesium (Mg) ,Phosphorous (P) ,Iron (Fe) ,Calcium (Ca), Sodium (Na), Manganese (Mn) ,Lead (Pb) & Cadmium (Cd) levels in spirullina powder of dry, wet

Address for correspondence

R. Paranthaman
Technical Officer (Laboratory),
Department of Food safety & quality Testing,
Indian Institute of Crop Processing Technology,
Ministry of food Processing Industries, Government of India,
Thanjavur- 613 005, Tamil Nadu, India
Email ; paranthhu@gmail.com

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and microwave digested using inductively coupled plasma optical emission spectrometry (ICP-OES).

2. MATERIAL & METHODS

The analysis of minerals in spirullina powder samples by using ICP-OES method [9-12]. All minerals and heavy metals measurements were carried out using the Perkin-Elmer optima ICP- OES (Model: OPTIMA2000DV, seial number: 080N3041701) and the ICP-OES operating conditions are listed in table 1.

2.1 ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) Analysis of Samples

All samples were analyzed in triplicates by ICP-OES Perkin-Elmer; model Optima™ 2000 DV, using winLab32 software for the analysis. The analytical measurements were made with a simultaneous perkin–Elmer ICP OES, model optima 2000DV, winLab32™, version 7.0 software equipped with a peristaltic pump, a cross-flow nebulizer (coupled to a ryton double pass spray chamber) and a ceramic central torch tube injector with an internal diameter of 2 mm. The operating parameters are listed in Table 1 . The wavelengths, measurement parameters and standards for each element are given in Table 2.

Table 1: Instrumental Conditions and Parameters
Parameter Setting

Injector	Alumina 2 mm i.d.
Sample tubing	Standard 0.76 mm i.d.
Drain tubing	Standard 1.14 mm i.d.
Quartz torch	Single slot
Sample capillary	PTFE 1 mm i.d.
Sample vials	Polypropylene
Source equilibrium delay	15 sec
Plasma aerosol type	Wet
Nebulizer start up	Instant
RF power:	1450
Nebulizer flow	0.7 L/min
Auxiliary flow	0.2 L/min
Plasma flow	15 L/min
Sample pump rate	1.5 mL/min
Plasma viewing:	Axial, radial
Processing mode	Peak area
Auto integration (min-max):	0.1-0.5 sec
Replicates	2
Background correction	1 or 2-point, manual

Table 2: Wavelengths, Measurement Parameters and Standards for each Element

Element	Wave length (nm)	Plasma (L/min)	Aux (L/min)	Neb (L/min)	power	View Mode	Calibration (mg/Kg)
Potassium (K)	769.896	15	0.2	0.7	1450	Radial	1 - 5
Magnesium (Mg)	279.077	15	0.2	0.8	1300	Radial	1 - 5
Phosphorous (P)	259.939	15	0.2	0.8	1300	Axial	1 - 5
Iron (Fe)	238.204	15	0.2	0.8	1300	Axial	1 - 5
Calcium (Ca)	393.366	15	0.2	0.8	1300	Radial	1 - 5
Sodium (Na)	589.592	15	0.2	0.8	1300	Axial	1 - 5
Manganese (Mn)	257.610	15	0.2	0.8	1300	Axial	1 - 5
Lead (pb)	283.306	15	0.2	0.8	1300	Axial	1 - 5
Cadmium (Cd)	228.802	15	0.2	0.8	1300	Axial	1 - 5

2.2 Analysis of certified reference material (CRM) of Minerals and calibration

A liquots of an ICP multielement standard solution (10 to 50 mg/L Merck) containing the analyzed elements (As, Hg, Se, Zn, P, Pb, Cd, Fe, Mn, Cr, Mg, Cu, Ca, Na and K) were used in the preparation of calibration solutions. Working standard solutions were prepared by dilution of the stock standard solutions to desired concentration in 1% HNO3.

2.3 Sample Preparation

The determination of metal contents was carried out using dry, wet and microwave digestions by inductively coupled plasma-optical emission spectrometry (ICP-OES).

2.3.1 Reagents and chemicals

All chemicals used throughout the experiments were of analytical- reagent grade (Merck, Darmstadt, Germany). HNO3 (65%), H2O2 (30%) and HCI (37%) were of analytical grade (E. Merck, India). All glassware and polyethylene bottles were kept overnight by soaking in 10% HNO3, and cleaned by

rinsing five times with distilled de-ionized Ultra High Quality water (Millipore, India)

2.3.2. Dry digestion

Spirullina powder samples (1.0 g) were placed into a high-form porcelain crucible. The furnace temperature was slowly increased to 450ºC. The samples were ashed for about 16 h until a white ash residue was obtained. The ashed sample was treated with 5 ml HNO3 (25% v/v) and the mixture, where necessary, was heated slowly to dissolve the residue. The solution filtered through whatman filter paper and then the sample was transferred to a 10 ml volumetric flask and made up to volume. Blanks were prepared in the same way as the sample, but omitting the sample. The determination of metal contents in this clear solution was carried out by ICP-OES. Three replicates (acid digests) were performed for each sample.

2.3.2. Wet digestion

For the digestion of Spirullina powder , the temperature was maintained at 130 ºC for 4 h during digestion of 1.0 g of sample with 6 ml HNO3 (65%) and 2 ml H2O2 (30%) mixtures on the hot plate until the solubilization of the sample was completed and then diluted to 10 ml with distilled water. The residue was filtered through whatman filter paper and then the sample was diluted to 10 mL with distilled water. Element contents of final solution were determined by ICP-OES. Blanks were prepared in the same way as the sample, but omitting the sample.

2.3.3. Microwave digestion

The Spirullina powder was digested using microwave digestor (Model: ETHOS One, Make – Millestone). A microwave assisted digestion procedure was carried out, in order to achieve a shorter digestion time. Weighed triplicate 0.25 g of certified samples and 1.0 g of real samples in reaction vessels (100 mL in capacity) directly, added to each flasks 8 mL of a freshly prepared mixture of concentrated HNO3–H2O2 (6:2, v/v) and stood for 10 min. Digestion conditions for the microwave system were applied as 2 min for 250 W, 2 min for 0W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min. After cooling, the resulting solutions were diluted up to 10 mL in volumetric flasks with 1 M HNO3.

3. RESULT AND DISCUSSIONS

The certified reference material (CRM) of Potassium (766.490) , Magnesium (280.271) ,Phosphorous (213.617) ,Iron (238.204) ,Calcium (393.366), Sodium (589.592), Manganese (257.610) ,Lead (220.353) & Cadmium (228.802) was analysed and calibrated. The ranges of the calibration curves (5 points) were selected to match the expected concentrations for all the elements of the sample studied by ICP-OES. The correlation coefficient r^2 obtained for all cases was 0.9999 and the calibration summary was given in table.3.

Table 3: Calibration summary

Element	Wave length (nm)	Standards	Equation	Intercept	Slope	Corr. Coef.
Potassium (K)	769.896	5	Linear	26619.4	22250	0.997165
Magnesium (Mg)	279.077	5	Linear	3648.9	569100	0.999985
Phosphorous (P)	259.939	5	Linear	61.5 227.7	34490	0.968270
Iron (Fe)	238.204	5	Linear	243.4 4988	4988	0.993525
Calcium (Ca)	393.366	5	Linear	10521.1	59980	0.925267
Sodium (Na)	589.592	5	Linear	378802.2	5307000	0.977538
Manganese (Mn)	257.610	5	Linear	126299.9	778500	0.973246
Lead (pb)	283.306	5	Linear	171.3	4054	0.998868
Cadmium (Cd)	228.802	5	Linear	9047.3	66660	0.982602

The wet, dry ashing and microwave digestion methods were performed for determination of minerals in spirullina powder samples given in table 4,5 & 6). Each of the methods given a clear digest, which was

diluted with 1 % nitric acid to 50 mL. These samples were analyzed in duplicates and calculated using certified reference material mineral standards.. The microwave digestion results showed that Potassium (977.5 mg/Kg) , Magnesium (202.2 mg/Kg) ,Phosphorous (6041 mg/Kg) ,Iron (36.45 mg/Kg) and Calcium (425.9 mg/Kg) and yield also higher than wet and dry ash digestion methods (Table 7). The achieved results are in good conformity with certified values. The accuracy of the method was evaluated by means of trace element determination in the standard reference material. Relative standard deviations (RSD) were found below 5%.

Table 4: Mean Data of elements in spirullina powder using microwave digestion method

Analyte	Intensity	Replicates	Conc. Units mg/Kg	Std. Dev.	Conc. Units mg/Kg	Std. Dev.	RSD
K 769.896	21779752.4	2	977.5	19.22	977.5	19.22	1.97%
Mg 279.077	115095914	2	202.2	3.13	202.2	3.13	1.55%
P 213.617	1375351.5	2	6041	142.5	6041	142.5	2.36%
Fe 238.204	179998.0	2	36.45	0.528	36.04	0.528	1.47%
Ca 317.933	21539355.0	2	425.9	11.46	358.9	11.46	3.19%
Na 589.592	27021280.2	2	5.163	0.1007	5.163	0.1007	1.95%
Mn 257.610	1428669.6	2	1.673	0.0661	1.673	0.0661	3.95%
Pb 220.353	765.5	2	0.147	0.0022	0.147	0.0022	1.49%
Cd 228.802	3592.7	2	- 0.082	0.0006	-0.082	0.0006	0.75%

Table 5: Mean Data of elements in spirullina powder using wet ashing method

Analyte	Intensity	Replicates	Conc. Units mg/Kg	Std.Dev.	Conc. Units mg/Kg	Std.Dev.	RSD%
K 766.490	3076912.3	2	137.1	3.32	137.1	3.32	2.42%
Mg 285.213	1459124.4	2	2.570	0.0780	2.570	0.0780	3.03%
P 213.617	229483.5	2	1008	24.3	1008	24.3	2.41%
Fe 238.204	59435.5	2	11.87	0.249	11.87	0.249	2.10%
Ca 317.933	7439172.7	2	123.8	6.16	123.8	6.16	4.98%
Na 589.592	5191450.6	2	1.050	0.0204	1.050	0.0204	1.94%
Mn 257.610	472062.2	2	0.444	0.0221	0.444	0.0221	4.98%
Pb 220.353	978.6	2	0.199	0.0045	0.199	0.0045	2.24%
Cd 228.802	1506.2	2	-0.113	0.0011	-0.113	0.0011	0.95%

Table 6: Mean Data of elements in spirullina powder using dry ashing method

Analyte	**Intensity**	**Replicates**	**Conc. Units** mg/L	**Std.Dev.**	**Conc. Units** mg/Kg	**Std.Dev.**	**RSD**%
K 766.490	11898752.5	2	533.5	13.73	533.5	13.73	2.57%
Mg 285.213	4804445.2	2	8.448	0.0522	8.448	0.0522	0.62%
P 213.617	686353.5	2	3015	62.3	3015	62.3	2.07%
Fe 238.204	182047.6	2	36.04	0.159	36.45	0.159	0.44%
Ca 317.933	25557693.9	2	358.9	1.85	425.9	1.85	0.43%
Na 589.592	18538487.1	2	3.565	0.0791	3.565	0.0791	2.22%
Mn 257.610	1523327.9	2	1.795	0.0259	1.795	0.0259	1.44%
Pb 220.353	1258.8	2	0.268	0.0101	0.268	0.0101	3.78%
Cd 228.802	2420.5	2	-0.099	0.0004	-0.099	0.0004	0.40%

Table 7: Result Summary

Elements	**Wave Length**	**Mean Data Concentrations (mg/Kg) (dry basis)**		
		Microwave	**Wet Ashing**	**Dry Ashing**
Potassium (K)	766.490	977.5	137.1	533.5
Magnesium (Mg)	280.271	202.2	2.570	8.448
Phosphorous (P)	213.617	6041	1008	3015
Iron (Fe)	238.204	36.45	11.87	36.04
Calcium (Ca)	393.366	425.9	123.8	358.9
Sodium (Na)	589.592	BDL	BDL	BDL
Manganese (Mn)	257.610	5.163	1.050	3.565
Lead (pb)	220.353	1.673	0.444	1.795
Cadmium (Cd)	228.802	0.199	0.147	0.268

*Below Detection Limit

4. CONCLUSION

The comparison of dry wet and microwave digestion methods showed no statistically significant differences in results obtained with these three methods. The results showed that dry and wet ashing procedures were slow and time consuming, so microwave digestion procedure was preferred. Microwave sample digestion is an accurate, simple and fast method for the ICP-OES determination of Potassium (K) , Magnesium (Mg) ,Phosphorous (P) ,Iron (Fe) ,Calcium (Ca), Sodium (Na), Manganese (Mn) ,Lead (Pb) & Cadmium (Cd) in spirullina powder samples. Spirullina powder was a very important human nutrient since their consumption has increased in recent years. Reviews clearly show that Spirulina has many therapeutic uses and great nutritional value. Therefore, results suggest that there good nutritive value and it can be concluded that Spirulina has a great scope in the field of human health and can be utilized for achieving food and nutritional security for nation.

5. Funding

This work was supported by Indian Institute of crop processing technology, Pudukottai Road, Thanjavur, Tamilnadu.

6. ACKNOWLEDGEMENTS

The authors would like to special thank **Dr. K. SINGARAVADIVEL (K. S. Vel),** Director, Indian Institute of Crop Processing Technology (IICPT), Ministry of Food Processing Industries, Govt. of India, Thanjavur-613 005 Tamil Nadu, for their support of this study.

7. REFERENCE

1. Henrikson, R. Earth Food Spirulina. How this remarkable blue-green algae can transform your health and our planet, Ronore Enterprises Inc., Laguna Beach, California;, 1989. pp 180.
2. Ciferri, O. *Microbiol. Rev.* 1983, *47*, 551-578.
3. Richmond, A. Spirulina. In *Micro-Algal Biotechnology*,Borowitzka, M. A. and Borowitzka, L. J. Eds., Cambridge University Press (1988).,
4. Schwartz, J.; Scklar, G.; Suda, D. *Cancer Nutrition* 1988, June.
5. Roughan, P. G. *J. Sci. Food Agric.* 1989, *47*, 85.
6. Cohen, Z.; Vonshak, A.; Richmond, A. *Phytochem.* 1987, *26*,2255-2258.
7. Mark F Mc Carty Clinical. Potential of *Spirulina*as: A source of phycocyanobilin. *Journal of Medicinal Food,* (2007). 10(4): 566-570.
8. Srilakshmi B (2001). *Food Science*. 2nd Edition. Delhi: New Age International Limited Publishers.
9. Huseyin Altundag and Mustafa Tuzen . Comparison of dry, wet and microwave digestion methods for the multielement determination in some dried fruit samples by ICP-OES. Food and Chemical Toxicology, 2011. 49, 2800–2807
10. U.S. EPA Method 200.7: "Determination of metals and trace elements in water and wastes by Inductively Coupled Plasma Optical Emission Spectrometry", Cincinnati, Ohio, U.S.
11. IS 3025 (part 2): 2004, ISO 11885:1996. "Water Quality-Determination of 33 Elements by Inductively Coupled Plasma Optical Emission Spectrometry".
12. PerkinElmer Application Library for ICP-OES.

How to cite this article: R. Paranthaman, R.Vidyalakshmi, S.Kumaravel; Optimization of digestion methods for mineral in spirullina by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES); J. Adv. Pharm. Edu. & Res. 2014: 4(4): 417-421.

Source of Support: Nil, **Conflict of Interest:** Nil