Journal of Food and Drug Analysis, Vol. 9, No. 3, 2001, Pages 178-182 藥物食品分析 第九卷 第三期

Inorganic Elements Determination for Algae/Spirulina Food Marketed in Taiwan

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(Received : February 13, 2001 ; Accepted : June 11, 2001)

ABSTRACT

The purpose of this study was to determine the inorganic elements in 8 commercial algae foods using INAA and ICP-AES methods. The results showed that the Na, Cl, and Al concentrations in green algae food are 1.5~7 times less than those in Spirulina food. Other heavy metal contents, including As, Cd, Cu, Hg, Pb, Cr, and Ni, were all within the allowed daily intake levels. The Spirulina food samples were found to contain more than 1 μg/g of As, which is the limitation level for green algae food samples, and one contained 15 μg/g of Pb. Since the concentrations of the inorganic elements in this study were not found to exceed the present regulation levels, they can be considered as safe food.

Key words: algae, Spirulina, inorganic element

INTRODUCTION

Algae, which is considered to be a natural food, is abundant in protein (50~65%), amino acids (more than 10 types), and chlorophyll (~5%)[1]. It also contains some trace elements[2], which are essential to the human body. Several brands of health food made of cultured green algae and Spirulina are now commercially available. These algae foods involve both trace elements and amino acids, which are readily absorbed and utilized by the human body. However, due to the cultured condition differences, trace element contents could vary in different brands of algae food. Those trace elements in a suitable range level could be beneficial to our body; while an overdose of trace elements could create an additional load on the human body and thus a detrimental effect could occur.

In general, the human body is composed of two groups of elements, namely common and trace elements[3]. Common elements account for up to 99% of human body constituents, including the four organic elements of O, C, H, N (96%), and the elements of Ca, P, S, K, Na, Cl, Mg etc (3%). Only 10^{-3}%~10^{-7}% trace elements exist in the human body. Because only trace amounts of trace elements exist, enhancing detection sensitivity could increase the detectable numbers of trace elements. Until now, more than 70 trace elements have been found. (Some significant trace elements are listed in Table 1 [3].)

It is known that an overdose of trace elements is harmful to health. It was reported in Japan that consuming cadmium contaminated rice could cause Itai-Itai disease[4]. Overdoses of lead can cause lead poisoning, resulting in intelligence retardation and slow reactions[5]. Arsenic could cause black-foot-disease[6]. Some evidence also reveals that insufficient trace elements could affect human health. For example, a lack of the elements Se, Ge, Zn, Fe, and Mn could have a detrimental effect on body metabolism and the development of the brain and bones[7].

In this study, the Instrumental Neutron Activation Analysis (INAA) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) methods were used to analyze the inorganic elements in 8 commercial algae foods. The main purpose of this study was to determine if the heavy metal contents in those health foods were higher than the tolerant levels. In addition, by analyzing the inorganic elements, the properties of algae food could also be characterized.

MATERIALS AND METHODS

I. *Materials*

Eight marketed algae food samples were randomly collected from chain stores such as WATSON'S, COSMED, or special contract stores. Six of the samples were green algae food (G1-G6) and two were Spirulina food (B1-B2). All samples were within the effective usage period according to the expiration date shown on the packages. Table 2 lists the daily intake correction factors, which were used as the calculation bases to correct the daily intake for the collected food samples, since the recommendation daily intakes varied for different brands.

II. *Detection Methods*

INAA is a non-destructive detection method and capable of simultaneously identifying and quantifying many inorganic elements without any chemical pre-treatment. Thus, the chance of sample contamination can be minimized. However, some elements such as lead, copper, cadmium, and mercury are either small in the reaction cross-section to neutrons or readily interfered with gamma-ray energies by other

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Journal of Food and Drug Analysis, Vol. 9, No. 3, 2001

Table 1. Human major composition elements

Common elements				Trace elements (weight percent: 5×10^{-3}–1.4×10^{-7}%)	
Organic	Weight percent	Elements	Weight percent	Essential (14-17 elements)	Maybe / Non essential (≥15 elements)
O	61 %	Ca	1.40 %	Fe	Rb
C	23 %	P	1.10 %	Zn	As
H	10 %	S	0.20 %	Cu	Si
N	2 %	K	0.20 %	Mn	Bi
		Na	0.14 %	Cr	Al
		Cl	0.12 %	Mo	B
		Mg	0.03 %	Co	Ti
				Se	Pb
				Ni	Cd
				V	•
				•	•

Table 2. Daily intake correction factors for various samples

Sample number	G1	G2	G3	G4	G5	G6	B1	B2
Correction factor	10.0	4.8	4.0	3.0	5.0	6.0	5.0	3.0

Table 3. Inorganic elements to be determined by INAA method

Irradiation time	Cooling time	Elements to be determined
Short (5 min.)	5 min.	Mg、Al、Cl、Ca、Ti、Mn
Long (20 hr.)	3 d.	Na、K、Br、As、La、Sm、Sr
	21 d.	Sc、Cr、Fe、Co、Zn、Rb、Se、Sr、Cs、Ba

elements, resulting in low detection sensitivity. Phosphorus (P) is likely to be activated to release β^-emitter ^{32}P. Therefore, the gamma-ray spectrometer is not suitable for P detection.

ICP-AES has been widely adopted in inorganic element detection. In this study, INAA was mostly used to detect the inorganic elements in algae food samples; while ICP-AES was used to detect those elements (lead, copper, phosphorus, cadmium, and mercury), which show less sensitivity to the INAA method. Furthermore, these two methods were mutually validated by the detection of potassium.

(I) *INAA Detection*

A Zero-Power Reactor (ZPRL) at Lungtan, Taiwan, was used to provide the neutron source for INAA detection. The maximum neutron flux in the reactor core was about 6.7×10^{11} n•cm^{-2}•sec^{-1}. Inside the reactor core, there exists an open area (7 cm × 7 cm) allowing for irradiating samples.

Algae food samples (0.8~1 g) were individually packed into a polyethylene plastic bag prior to irradiation. The reference standards (peach leaves SRM1547 and coal fly ash SRM1633b), which were used as the quantification standards, were purchased from the National Institute of Standards and Technology (NIST).

Test samples were exposed to neutron beams for 5 or 20 hours depending on the elements to be analyzed. After an appropriate time of cooling, the activated samples were analyzed with a gamma-ray spectrometer to identify and quantify the inorganic elements in test samples. The inorganic elements, which are intended to be determined by the INAA method, are listed in Table 3.

(II) *ICP-AES Detection*

Five inorganic elements including lead (Pb), copper (Cu), phosphorus (P), cadmium (Cd), and mercury (Hg) were analyzed using ICP-AES (Leeman model SP1000) in this study. The reagents purchased from E. Merck Co. were used for the preparation of standard solutions. Test samples (~1g) were digested with a mixture of nitric acid (5 mL) and sulfuric acid (3 mL) and heated with an electric plate prior to analysis.

To determine if the sample matrix could interfere with the analyte, the inorganic elements listed in Table 4 were spiked into the test samples of green algae G5 and Spirulina B1 and tested for recovery. The relative errors of recovery for spiked elements were all within 10% as shown in Table 4.

RESULTS

The inorganic element contents in algae food analyzed either by INAA or ICP-AES were designated to be mg/g or μg/g. Because the recommend daily intake levels were different among those test samples, the inorganic contents were converted to daily intake concentrations (mg/d) by multiplying a factor as listed in Table 2 in order to more effectively reflect the actual daily intake levels.

Table 5 and 6 lists the daily intake levels of common and trace elements expressed by mg/d and μg/g. Data were mostly generated by using INAA except for the data with parentheses, which were generated by using ICP-AES. The potassium level in test samples was analyzed by both methods, and the results generated from these two methods were consistent. Titanium was not found in most of samples, except for sample G4 as listed in Table 6.

The error percentage for detection of K, Na, Cl, Mg, P, Mn, Cu, Br, and Pb were within 10%, while for Fe, Al, Cr, Co, Sc, Rb, and Ba detection were 10~20%, and for Ca, Sr, As, and Zn were 20~30%. Sulfur content in all test samples was lower than the detection limit of the instrument. All the errors, including statistical and systematic errors, were estimated repeatedly after being analyzed 4 times.

Table 4. ICP-AES recovery tests

Element	Addition (μg)	Measurement (μg)	Recovery (%)	Addition (μg)	Measurement (μg)	Recovery(%)
		(G5)			(B1)	
Cu	5.0	4.7	94	5.0	4.6	92
Pb	10.0	11.0	110	10.0	9.1	91
Cd	5.0	5.4	108	5.0	4.7	94
P	10000	9320	93	10000	10200	102
K	8000	8420	105	8000	7920	99
Hg	5.0	4.5	90	5.0	4.5	90

Table 5. Common element daily intake[a] for this study

Common	Daily Intake[b] (mg/daily)							
Element	G1	G2	G3	G4	G5	G6	B1	B2
K	77.8 (64.0)	43.0 (43.2)	42.4 (32.8)	7.2 (5.1)	44.4 (35.5)	50.9 (36.6)	54.5 (45.5)	46.2 (42.0)
Na	3.2	1.0	1.2	0.6	1.0	2.3	18.7	29.7
Ca	49.9	36.9	19.7	7.5	22.6	14.8	20.3	4.4
Cl	1.8	1.5	0.6	1.2	1.7	1.2	14.9	16.5
Mg	24.3	17.2	11.7	2.9	15.7	10.9	20.1	16.0
P	(118.0)	(85.0)	(62.4)	(13.2)	(78.5)	(41.4)	(40.0)	(27.0)
S	(< 100)	(< 48)	(< 40)	(< 30)	(< 50)	(< 60)	(< 50)	(< 30)

[a]Data with parenthesis are measured with ICP-AES otherwise INAA will be prevailed.
[b]Estimated errors: ~10% for K 、Na 、Cl 、Mg 、P
20~30% for Ca.

Table 6. Trace element daily intake for this study

Trace	Daily intake[a] (μg/daily)							
element	G1	G2	G3	G4	G5	G6	B1	B2
Fe	5957.0	5260.8	4859.6	12768	7140.5	6794.4	4318.5	1188.0
Al	1340.0	700.8	732.0	294.0	595.0	270.0	2080.0	7097.0
Mn	275.0	258.7	309.6	81.3	229.0	1342.8	265.5	90.0
Zn	507.0	158.4	90.0	19.1	120.5	59.6	236.5	62.4
Cu	(120.0)	(30.6)	(32.7)	(10.4)	(24.5)	(41.6)	(49.6)	(36.0)
Cr	6.9	3.8	2.3	2.0	3.1	2.6	3.2	2.0
Br	4.4	14.9	1.4	6.0	18.5	4.7	43.5	11.9
Sr	392.0	115.2	156.8	<33.4	108.0	87.6	203.0	79.2
Ba	117.6	64.9	45.7	65.1	161.5	162.3	86.5	<30.0
Co	0.6	2.4	1.4	1.0	3.0	0.8	1.8	1.1
Rb	12.7	15.0	6.6	<7.6	14.4	8.4	33.0	3.5
Sc	0.1	0.2	0.1	0.1	0.2	0.1	0.7	0.1
La	<1.4	3.7	0.4	0.5	4.6	<0.75	<2.5	<1.41
Se	<0.08	0.1	<0.04	0.1	0.2	0.1	<0.05	<0.02
Sm	<0.3	0.6	<0.14	0.1	0.7	<0.13	0.3	<0.135
As	<3.5	4.6	2.1	1.4	2.4	3.6	9.9	<3.84
Hg	<0.3	<0.14	<0.12	<0.09	<0.15	<0.18	0.15	<0.09
Pb	(<5.0)	(<2.4)	(<2.0)	(<1.5)	(<2.5)	(<3.0)	(76.0)	(16.8)
Ti				3924.9				

[a]Estimated errors: ~10% for Br 、Mn 、Cu 、Pb
10~20% for Fe 、Al 、Cr 、Sc 、Co 、Rb 、Ba 、La 、Se 、Sm
20~30% for As 、Sr 、Zn.

DISCUSSION

I. *Heavy Metal Contents*

In general, heavy metals are the metals which belong to the transition elements with higher specific gravity. However, only 7 of them, Cd, Cu, Cr, Ni, Hg, Pb, and Zn, have received more attention, due to their detrimental effect on health and the possibility of food contamination. Arsenic carries similar properties to the heavy metals, although it is categorized as a none-heavy metal element. Therefore, the results of analyzing the above 8 elements are discussed as follows.

The recommend daily intake heavy metal elements for humans are listed in Table 7[(7)]. As compared to the results listed in Table 6, all the contents of heavy metal elements in this study were far below the recommend daily intake levels (Table 7). The recommend daily intake levels listed in Table 7 include all the food sources. However, the ratio of algae food consumption is far less than other food consumption. Comparing the data listed in Table 6 to those listed in Table

7, most of the elements in the algae food samples showed less than 10% of recommend daily intake, except for Cr in G1 and Pb in B1 samples, which showed 13.8% and 25.3% of recommend daily intake, respectively.

According to the heavy metal element regulation announced by the Department of Health, ROC, arsenic in green algae and Spirulina food is limited to 1 and 2 μg/g, respectively, and heavy metal (based on Pb element) is restricted to 5 and 20 μg/g, respectively. The heavy metal concentrations for the test samples are summarized in Table 8. Arsenic contents in green algae food samples were all within the regulation range (1 μg/g). Although it showed higher arsenic contents in Spirulina food samples, those concentrations were still within the regulation range (2 μg/g). The spectro-photometric method is the official method for the detection of lead, which is designated to be the total heavy metal contents. Thus, the individual heavy metal contents determined in this study can not be used to compare the total heavy metal contents detected by a spectro-photometric method.

II. *Common and Trace Elements*

The contents of common/trace elements labeling for marketed algae food are quite different and selective, depending on the brands or manufactures. Potassium (K), calcium (Ca), and iron (Fe), for example, are the elements frequently selected for algae food labeling. The labeling contents could only be used as a reference since the test results were different from the label contents.

(I) *Common Element Contents*

The contents of phosphorus (P), sulfur (S), potassium (K), and calcium (Ca) in algae food were found to be higher than the other common elements. This result reveals a common property existing in this kind of health food. In terms of the common element contents, a significant difference was observed between green algae and Spirulina food. The sodium (Na) and chloride (Cl) contents in Spirulina food were even 5 times higher than those in green algae food. This could be due to fact that the Spirulina was cultured in seawater.

Table 7. Recommend daily intake heavy metal elements

Element	As	Pb	Zn	Cu	Hg	Cd	Cr	Ni
Daily intake (μg/daily)	100	300	13000	2500	10	40	50	400

(II) *Trace Element Contents*

The major trace elements in algae food included iron (Fe), zinc (Zn), aluminum (Al), copper (Cu), and manganese (Mn), and their contents ranged from tens to ten thousands microgram (µg). The results of our study indicated that the contents of the above 5 elements in all test samples, G1-G6 and B1-B2 as listed in Table 9, were far below the recommend daily intake (RDI) level or recommend safety contents reported by some literature. These results are reasonable because RDI counts the elements in all the food being taken daily; while algae food merely represents one of those foods.

The RDI percentage for iron in both group G and B were significantly higher than those for other elements. This result is in accordance with a trait of green algae food that is usually declared with high iron content. The result as shown in Table 9 also reveals that Group B food was rich in aluminum (Al), indicating the aluminum content in Spirulina food was much higher than that in green algae food.

It has been reported that algae food is also abundant in strontium (Sr) and barium (Ba). A well-known property of algae is its strontium absorption ability. This trait had been used to absorb radioactive strontium for treatment of nuclear waste with high radioactivity[8].

The results also showed that G4 food sample contained high concentration of titanium (Table 6). G4 food sample was the only test sample packed in capsule form. After analysis, we found that the titanium in G4 food mainly came from the

Table 8. Heavy metal concentration for this study(G1-G6, B1-B2) unit: μg/g

Sample	G1	G2	G3	G4	G5	G6	B1	B2
As	<0.35	1.0	0.5	0.5	0.5	0.6	2.0	<1.28
Pb	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	15.2	5.6
Zn	50.7	33.0	22.5	6.4	24.1	9.9	47.3	20.8
Cu	12.0	6.4	8.2	3.5	4.9	6.9	9.9	12.0
Hg	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03	<0.03
Cd	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cr	0.7	0.8	0.6	0.7	0.6	0.4	0.6	0.7

Table 9. RDI and recommend safety contents for certain major trace elements

	RDI[a]	G1 - G6[b]		B1 - B2[b]	
	(mg/daily)	mg/daily	RDI Percent (%)	mg/daily	RDI Percent (%)
Fe	16	4.9-12.8	(30.6-80) %	1.2-4.3	(7.5-26.9) %
Zn	13	0.02-0.51	(0.2-3.9) %	0.06-0.24	(0.5-1.9) %
Al	10	0.27-1.34	(2.7-13.4) %	2.1-7.1	(21-71) %
Cu	2.5	0.01-0.12	(0.4-4.8) %	0.04-0.05	(1.6-2) %
Mn	4	0.08-1.34	(2-33.5) %	0.09-0.27	(2.2-6.8) %

[a]Ref. 7.
[b]This study (Table 6).

capsule. The purpose of using titanium (titanium dioxide) is to provide a white color so that the capsule becomes opaque. Basically, titanium is not harmful to human health since it does not easily accumulate in human body.

CONCLUSIONS

The inorganic elements in 8 marketed algae food were identified and quantified in this study. Results showed that the contents of Na, Cl, and Pb in green algae food samples were significantly lower than those in spirulina food samples. The contents of As, Cd, Cu, Hg, Pb, Cr, and Ni in all test algae food samples were all within the daily intake levels. Although the As content in Spirulina food and Pb content in B1 algae food sample were higher than others, those concentrations were found not to exceed the regulation levels. Therefore, all the test samples were considered to be safe food.

ACKNOWLEDGEMENTS

We would like to thank Mr. Sheng-Man Tsao, Mr. Chao-Dan Lee, and Mr. Yu-Chih Hung of the Institute of Nuclear Energy Research for the Zero-power reactor operation. We are also very grateful to division head Mr. Chung-Jyi Wu. Without his support, the initiation of this research would be impossible. We thank Dr. C. W. Chen for his translation work.

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數種市售藻類食品內無機元素之含量分析

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（收稿：February 13, 2001 ；接受：June 11, 2001）

摘　要

本文敘述利用儀器中子活化分析法及感應耦合電漿原子光譜儀法，測定8種市售藻類食品內無機元素的含量。結果顯示綠藻類食品中所含的鈉（Na）、氯（Cl）、鋁（Al）比藍／螺旋藻少約1.5-7倍，但兩種藻類食品中重金屬如砷（As）、鎘（Cd）、銅（Cu）、汞（Hg）、鉛（Pb）、鉻（Cr）、鎳（Ni）等，則均在人們正常每日攝取量的範圍內。雖然藍／螺旋藻所含的砷超過綠藻的法定限量標準（1 μg/g），以及一家廠牌（B1）檢測出含有鉛（15 μg/g），但由於並未逾越該類食品現有法規之限量規定，故仍應屬安全食品。

關鍵詞：綠藻，藍/螺旋藻，無機元素