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Determination of Phosphorus in Foods by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

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Abstract

The analytical method for the determination of phosphorus in foods was validated by inductively coupled plasma optical emission spectrometry (ICP-OES) in terms of precision, accuracy, recovery efficiency and linearity. Regression analysis revealed good correlation coefficient, higher than 0.999. Recovery efficiencies of the minerals ranged from 90.36% to 110.63%, and the limit of detection (LOD) and the limit of quantification (LOQ) were 0.0745 mg/kg and 0.2482 mg/kg, respectively. The value of inter-day and intra-day ranged from 1.43 to 3.23% and from 0.40 to 1.77%. The recovery efficiencies ranged from 97.8 to 110.6%. The method was also compared with Molybdenum blue colorimetric method using certified and statistically significant difference was also not observed in the between two different analytical methods. The ICP-OES method was applied to phosphorus determination in commonly consumed foods. The obtained results suggest that the method verified in the present study may be used as an official analytical method for clear understanding of phosphorus database for national health promotion.

Key words: phosphorus, foods, ICP-OES, Molybdenum blue colorimetric, microwave digestion

Introduction

Phosphorus (P) is an important component of foods as a mineral nutrient. In human body, most of the phosphorus content is involved in the formation of bones and teeth. The rest is found in the cells and other tissues (Stover *et al*., 1994; Joyce, 1999). The Recommended Dietary Allowance (RDA) of phosphorus is 1250 mg/day for youth age 9 to 18 years old, and 700 mg/day for adults 19 years old and older (Stover *et al*., 1994). Sufficient intake of the phosphorus is necessary to make an appropriate balance with calcium, which is related to prevent bone diseases such as osteoporosis and osteopenia (Heaney *et al*., 2004). One recent study demonstrated that ideal ratio of calcium to phosphorus improved bone mineralization and turnover with proper intestinal calcium and phosphorus absorption (Ritsuko *et al*., 2003). It was also reported that women over 60 years old consumes less than 70 percent of RDA of phosphorus and osteoporosis is likely caused by their low intake (Heaney *et al*., 2004).

The dietary phosphorus intake is easily available in many typical food sources. The main sources are the protein rich foods. Meats, fish, milk, dairy, eggs and cereals naturally contain very high levels of phosphorus. Fruits and vegetable are low in phosphorus (Jaime, 2007). Moreover, the addition of phosphorus during food processing and use of phosphorus-containing dietary supplement have increased the daily intake of the mineral (Bell *et al*., 1977; Uribarri *et al*., 2003). However, excessive intake of phosphorus leads to homeostatic imbalance, cardiovascular and severe kidney disease (Jastrzebska *et al.,* 2003). In particular, the dietary intake of phosphorus is usually restricted for chronic kidney disease (CKD) patients based on prescription of protein-controlled diet (0.9-1.0 g/kg/day) (Jaime, 2007). Recently, the information about amount of phosphorus obtained from several food sources has received much attention as the required or limited nutrient. The precise determination of phosphorus in foods is very essential to normal consumer as well as the patients.

Many analytical methods have been developed for the determination of phosphorus in different type of matrixes such as foods, plants, soils, water and fertilizers (Kocman *et al*.,

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1991; Guohui *et al*., 1995; Belikov *et al*., 1999; Weimin *et al*., 2002; Jastrzebska *et al*., 2003; Ranđel *et al*., 2007;). The most commonly used analytical method for phosphorus determination is a spectrophotometric method based on Molybdenum blue along with the technique available to small sample amounts of any matrix (HARRIS, 1954; AOAC, 1990; Ranđel *et al*., 2007). In recent years, instrumental methods including, X-ray fluorescence (Guohui *et al*., 1995; Belikov *et al*., 1999; Jastrzebska *et al*., 2003), inductively coupled plasma with optical emission spectrometry (ICP-OES) (Polidori *et al*., 2007; Raghad *et al*., 2013), ICP with mass spectrometry (MS) (Sabine *et al*., 2003; Cozzolino *et al*., 2008), ICP with atomic emission spectrometry (AES) (Weimin *et al*., 2002), capillary electrophoresis (Stover, 1999; Dušek *et al*., 2003) and ion chromatography (Stover *et al*., 1994) have been gradually developed as the modification methods. It is well known that ICP-OES method is less time consuming and more precise procedure in comparison with those of Molybdenum blue technique. On the other hand, by ICP-OES method, only a little information on the amount of phosphorus obtained from food sources is available today.

In the present study, ICP-OES method was carried out to determine phosphorus in several foods. In order to verify the accuracy of the analytical method recovery studies, certified reference materials were used and the obtained results were compared with those of Molybdenum blue method.

Materials and Methods

Chemicals and Materials

Standard solution of phosphorus (100 mg/L) was purchased from AccuStandard, Inc. (New Haven, CT, USA) The standard solution curve was prepared in 1, 5, 25 and 100 μg/mL concentrations by diluting a standard solution (100 mg/L) with deionized water. Standard Reference Material (SRM) 1849a and 1548 were purchased from national institute of standards & technology (NIST, Gaithersburg, MD, USA) that were used for the infant/adult nutritional formula and meat homogenate. LGC standards (LGC Group, Middlesex, UK) 7105 and 7104 were used for rice pudding and sterilized cream. Electronic grade hydrochloric acid (35%), nitric acid and hydrogen peroxide were purchased from Fine Chem. (Seoul, Korea). Also, several kinds of cereal grains, starches, sugars, legumes, nuts, seeds, vegetables, mushrooms, fruits, meats, oils, teas, and seasonings were purchased from Rural Development Administration (Jeonju, Korea).

Dry ash procedure for sample preparation of phosphorus analysis

This procedure is offered for sample preparation to determine mineral nutrition by Ministry of Food and Drug Safety (MFDS, 2013). Four Certified Reference Materials (CRM) samples (3 g) were placed on each crucible. After carbonization, the crucible was placed in muffle furnace at 550°C until ash residues are light gray. The crucible was cooled to room temperature. A small amount of water and 10 mL HCl (12 N) were added into the crucible and then dried on hot water bath. The residues were boiled after adding 10 mL of HCl (12 N) and then filtered into 100 mL volumetric flask. The insoluble residues and used filter paper were transferred into the crucible and then repeated using the same procedure of drying ash as the tested samples. A small amount of water and 2 mL HCl (12 N) were added into the crucible and then each sample was diluted with 5 mL water. The sample was heated in water bath and filtered into 100 mL volumetric flask. The sample solution was adjusted to exactly 100 mL in volume with water and analyzed for the determination of phosphorus by molybdenum blue colorimetric and ICP-OES method.

Microwave digestion procedure for sample preparation of phosphorus analysis

This procedure is offered for sample preparation to determine mineral nutrition by Ministry of Food and Drug Safety (MFDS, 2013). Aliquots of each sample (1 g) were accurately weighed in a digestion vessel. The concentrated nitric acid (8 mL) and hydrogen peroxide (2 mL) were added in the digestion vessels. The vessels were placed in the microwave oven digestion system (QWAVE 1000, Questron Technologies, Mississauga, Canada) at 180°C, with 15 min ramp to temperature, at maximum power of 1000 W, for 30 min. After cooling for 15min, the digested samples were adjusted up to 50 mL with Milli-Q system water (Millipore, Bedford, MA, USA) before phosphorus analysis.

Molybdenum blue colorimetric method

This spectrophotometric method is provided for phosphorus determination by Ministry of Food and Drug Safety (MFDS, 2013). The sample solution (1 mL) obtained from dry ash and microwave digestion procedures were transferred into a 25 mL volumetric flask. Two mL standard solution was added into another 25 mL volumetric flask. The aqueous solutions in two volumetric flasks were mixed with 2 mL ammonium molybdate solution and then allowed to stand at room temperature for 1 h.

After adding hydroquinone solution (2 mL) and sodium sulfite solution (2 mL) into each 25 mL volumetric flask, the sample solution was mixed with 25 mL water. After 30 min, absorbance of each solution was measured at 650 nm using a UV-visible spectrophotometer. The blank test was carried out in the same manner as the treated sample.

Inductively coupled plasma optical emission spectrometry (ICP-OES) method

Approximately 10 mL sample solution obtained from dry ash and microwave digestion procedures were transferred into a 50 mL volumetric flask and then diluted to median value in a standard curve with deionized water. The solution was analyzed for phosphorus using a ICP-OES (ACTIVA, Horiba, Jobin Yvon, France). The standard calibration curve for phosphorus was prepared using different concentration of standard phosphorus in deionized water (1, 5, 25 and 100 μg/mL), and each solution was injected into ICP-OES (Table 1).

Results and Discussion

Evaluation of method performance by ICP-OES

The validation of ICP-OES method for determination of phosphorus is listed in Table 2. A linear calibration curve for phosphorus in the range of 1 to 100 μg/mL was obtained with satisfactory correlation coefficient of 0.9999 and the values were greater than 0.9817 of previous work by colorimetric method including molybdenum blue colorimetric method (Aneta, 2009). The limit of detection (LOD) and limit of quantification (LOQ) values were estimated at an SD/b ratio of 3 and 10. The SD and b describe the standard deviation of the intercept and slope of regression line, respectively. The LOD and LOQ were 0.0745 mg/kg and 0.2482 mg/kg, respectively. The repeatability and recovery efficiency were measured by microwave digestion procedure using NIST CRM 1849a (0.08, 0.25, and 1 g) in Table 3. In the case of inter-day, the results ranged from 1.43 to 3.23 % (RSDs). The values for the intra-day ranged from 0.40 to 1.77 % (RSDs). The recovery efficiency (%) was calculated by using following equation.

Recovery (%) = $(C_t/C_a)\times100$

C_t is experimentally determined concentration of phosphorus found in CRMs and C_a is officially provided concentration of phosphorus in CRMs by NIST. The recovery efficiencies ranged from 95.8 to 110.6 %. From these results in the present study, the repeatability and recovery for ICP-OES was acceptable according to the guidelines of EU (The European Union) and FDA (FDA, 2001; The European Union On-line, 2006).

Table 1. Instrumental parameters of ICP-OES for determination of phosphorus

Parameter	Operating condition
RF Power	1000 W
Nebulizer	Seaspray
Nebulizer gas flow	1.17 L /min
Plasma gas flow	12 L/min
Sheath gas flow	0.2 L/min
Nebulizer pressure	2.80 bar
Normal speed of pump	20 rpm
Wavelength	P 213.618 nm

Table 2. Evaluation of the ICP-OES methods

Element	Linear range (mg/kg)	Correlation coefficient (r)	LOD (mg/kg)	LOQ (mg/kg)
Phosphorus	1-100	0.9999	0.0745	0.2482

Table 3. Evaluation of the ICP-OES methods

Element	Weight (g/50 mL)	RSD (%)		Recovery (%)
		Intra-day (n=3)	Inter-day (n=12)	
Phosphorus	0.08	1.77	3.00	95.75±2.22
	0.25	0.40	1.43	100.71±0.96
	1.0	0.77	3.23	110.63±3.39

Comparison of phosphorus determination between ICP-OES and Molybdenum blue colorimetric method

Table 4 shows the amounts of phosphorus in CRMs (Certified material samples) by the dry ash procedure using the ICP-OES and molybdenum blue colorimetric method. The determined values were compared to the reference results. Recovery efficiency of phosphorus ranged from 81.5 to 118.1% for molybdenum blue colorimetric method and 86.6 to 120.5 % for ICP-OES method from all CRMs tested in this study. Moreover, the t-test at 95 % confidence level was used for the comparison between the amounts obtained from between ICP-OES and molybdenum blue colorimetric method. The result of t-test revealed no statistically significant difference for the amount of phosphorus obtained by two different methods. For dry ashing method of phosphorus analysis, MFDS offers 550°C of the temperature condition as the official method. The different recovery efficiency with dry ashing method might be affected by various kinds of CRMs and temperature conditions, however the recovery efficiency was similarly obtained and satisfactory according to the guidelines of EU (The European Union) and FDA (FDA, 2001; The European Union On-line, 2006).

Table 4. Phosphorus determination in certified materials compared with ICP-OES and Molybdenum blue colorimetric method using dry digestion procedure

Temperature (°C)	CRMs	Molybdenum blue colorimetric method		Dry ashing method (ICP-OES)	
		Measured (mg/kg, mean±SD)	Recovery (%)	Measured (mg/kg, mean±SD)	Recovery (%)
350°C	SRM 1849a	3,517.43±98.78	88.16	4,295.83±22.26	107.66
	SRM 1546	1,246.81±34.68	81.49	1,325.48±60.53	86.63
	LGC 7105	408.51±145.50	118.07	369.08±8.44	106.67
	LGC 7104	825.62±36.68	100.32	942.23±22.41	114.49
450°C	SRM 1849a	3,794.25±113.68	95.09	4,555.51±64.11	114.17
	SRM 1546	1,465.49±68.67	95.78	1,761.79±41.80	115.15
	LGC 7105	347.30±66.26	100.38	362.14±6.76	104.66
	LGC 7104	863.78±35.57	104.96	992.03±41.91	120.54
550°C	SRM 1849a	3,936.61±9.88	98.66	4,096.96±36.30	102.68
	SRM 1546	1,527.54±29.17	99.84	1,613.39±15.22	105.45
	LGC 7105	393.16±10.16	113.63	348.19±1.18	100.63
	LGC 7104	885.97±13.48	107.65	848.93±13.91	103.15
650°C	SRM 1849a	3,456.76±36.18	86.64	4,479.12±21.10	112.26
	SRM 1546	1,498.93±57.95	97.97	1,718.19±47.56	112.30
	LGC 7105	317.64±13.07	91.80	359.67±7.61	103.95
	LGC 7104	757.86±12.02	92.09	897.30±10.26	109.03
t-test				-0.45 (2.05)	

In addition, recovery efficiency and the amounts of phosphorus from CRMs using microwave digestion procedure by ICP-OES and molybdenum blue colorimetric method is shown in Table 5. Recovery efficiency of phosphorus ranged from 83.1 to 111.6% for molybdenum blue colorimetric method and 95.6 to 112.1% for ICP-OES method from all CRMs tested in this study. Statistically significant difference was also not observed in the between the amounts of phosphorus obtained by two different methods. Based on the results obtained, it can be suggested that two different procedures are in agreement and the proposed ICP-OES method is also well-suited for phosphorus analysis. It is known that analysis of phosphorus by ICP-OES is relatively simpler and faster than traditional procedures such as colorimetric determination having the limited stability of reducing agents and slow rate for color formation (Weimin *et al*., 2002). The main advantage of ICP-OES is low LOD and it is available to determine trace amounts of phosphorus (Raghad *et al*., 2013). Moreover, the phosphorus determination by the ICP-OES in SRM 1849a was compared with dry ashing and microwave digestion method. Recovery efficiency of phosphorus ranged from 102.7 to 114.2% for dry ashing method and 95.8 to 110.6 % for microwave digestion method from SRM 1849a tested in this study. The difference was not significantly observed in the between the amounts of phosphorus obtained by two different methods.

Application of ICP-OES method for phosphorus determination in food samples

The determination of phosphorus in selected foods by ICP-

Table 5. Phosphorus determination in certified materials compared with ICP-OES and Molybdenum blue colorimetric method using microwave digestion procedure

CRMs	Molybdenum blue colorimetric method		Microwave method (ICP-OES)	
	Measured (mg/kg, mean±SD)	Recovery (%)	Measured (mg/kg, mean±SD)	Recovery (%)
SRM 1849a	4,451.18±56.90	111.56	4,376.60±96.69	109.69
SRM 1546	1,400.09±35.48	91.51	1,656.24±44.97	108.25
LGC 7105	369.52±17.55	106.80	364.06±23.33	105.22
LGC 7104	900.81±16.39	109.45	891.58±44.70	108.33
SRM 1548a	2,896.02±64.75	83.08	3,331.47±54.02	95.57
SRM 2385	314.27±11.54	97.09	362.81±5.51	112.08
t-test			-0.03 (2.45)	

Table 6. Phosphorus content in commonly consumed foods

Food type		N	Content (mg/100 g)		
Food species	Food sample		Min	Max	Mean±SD
Cereal grains	Cereal	7	29.90	321.40	142.57±3.05
	Biscuit	2	92.15	113.50	102.82±1.19
	Cookie	3	87.29	112.27	103.28±2.11
	Snack	3	40.66	193.19	106.01±5.55
	Cracker	3	113.21	142.48	127.76±1.84
	Pie	2	57.69	96.51	77.10±1.14
	wheat flour	6	82.70	329.88	143.71±1.02
Starches	Potato	17	32.98	474.14	84.00±1.24
	Sweet potato	6	46.54	63.22	54.23±0.63
Sugars	Sugar	3	0.00	1.15	0.38±0.05
	Candy	4	8.56	49.61	25.52±0.77
	Honey	4	2.90	9.04	7.09±0.05
Legumes	Pea	2	173.70	174.14	173.92±0.48
	kidney bean	2	270.07	280.65	275.36±3.44
	Mung beans	2	225.38	440.81	333.10±0.12
Nuts and seeds	Almond	3	522.71	554.95	539.91±4.61
	Sesame seed	2	584.37	746.41	665.39±10.42
Vegetables	Sesame leaf	3	72.06	82.53	77.31±0.15
	Carrot	2	38.97	42.06	40.52±0.16
	Green pepper	4	25.11	29.15	27.59±0.16
	Paprika	6	21.91	27.04	25.25±0.41
	Broccolini	2	60.48	67.71	64.09±0.02
	Red pepper	5	21.22	447.27	129.20±0.94
	Pumpkin	2	53.56	56.34	54.95±0.31
Mushrooms	Mushroom	5	82.90	114.69	95.43±0.72
Fruits	Kiwi	2	26.71	33.11	29.91±0.30
	Apple	2	7.99	8.59	8.29±0.08
	Grape	7	10.79	20.26	15.67±0.14
	Papaya	4	14.79	17.50	16.57±0.08
Meats	Goat	2	151.86	185.60	168.73±2.60
Seaweed	Seaweed	7	166.67	224.04	182.18±3.30
Oils	Soy oil	2	99.10	170.79	134.95±0.86
Teas	Green Tea	2	278.55	401.54	340.05±3.52
Seasonings	Soy sauce	2	46.26	145.46	95.86±1.24

OES was investigated and the obtained results are listed in Table 6. This procedure was carried out by microwave digestion, simple for sample preparation. Among the commonly consumed food species, cereal grains, starches, sugars, legumes, nut, seeds, vegetables, mushrooms, fruits, meats, seaweed, oils, teas and seasonings was estimated the amounts of phosphorus. From the obtained results in the present study, sugars exhibited the lowest level of total phosphorus the amounts of phosphorus, which ranged from 0.38±0.05 to 25.52±0.77 mg/100 g. The highest level of phosphorus in nuts and seeds was found in the greatest amount ranged from 539.91±4.61 to 665.39±10.42 mg/100 g, followed by legumes from 173.92±0.48 to 333.10 ± 0.12 mg/100 g. In the case of teas, see weeds, meats and oils the amounts of phosphorus was 340.05±3.52, 182.18±3.30 mg/100 g, 168.73±2.60 and 134.95± 0.86 mg/100 g, respectively. In the case of cereal grains, starches and vegetables, the phosphorus was ranged from 77.10±1.14 to 143.71±1.02 mg/100 g, from 54.23±0.63 to 84.00±1.24 and from 25.25±0.41 to 129.20±0.94 mg/100 g, respectively. In the case of mushrooms and seasonings, the amounts of phosphorus were 95.43±0.72 and 95.86±1.24 mg/ 100 g, respectively. Previous reports also indicated that the highest amounts of phosphorus were found in nuts from 345.65 to 472.62 mg/100 g, followed by dairy from 137.97 to 433.18 mg/100 g (Jaime, 2007). The amounts of phosphorus were not consistent with the previous results from the several foods. However, the phosphorus contents in foods can be different by several factors of cultivation, harvesting times and storage conditions and industrial processing. By the proposed ICP-OES method in this study, the estimation for phosphorus in commonly consumed foods may be valuable sources for awareness of the importance of appropriate phosphorus intake for bone health.

Conclusion

The ICP-OES showed great potential as an alternative method to current official method of molybdenum blue colorimetric method. The phosphorus determination by the ICP-OES in the certified materials was acceptable according to the guidelines of EU and FDA in terms of the precision and accuracy. In comparison to Molybdenum blue colorimetric method, the ICP-OES method provide simple and less time consuming as well as better linearity. Therefore ICP-OES can be applied to phosphorus determination in many foods as an efficient and suitable technique. According to different conditions for the cultivation or manufacture of foods, the clear estimation of phosphorus in foods and dietary supplements will be studied in the future work for most consumers or CKD patients.

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