

Original Article

Some Notes on Our Food Science Experiment II

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The opportunities for chemical experiments by our second year students are considerable; they had already experienced Food Science Experiment I which included many experiments in the field of organic food chemistry. In addition, they had already studied basic chemical experiments. Given this background, the experience and knowledge of chemistry among our sophomores seems to be good; though, it is still better to choose nontoxic chemicals, or to select less dangerous procedures or plans as much as possible. Consequently, both appreciation of safety in the laboratory and the least pollution of the environment are important considerations.

In this experiment, the determination of mineral nutrients in foodstuffs is an important subject. A dry incineration method was chosen and used as a pretreatment for inorganic analysis. This substituted for wet digestion which is a general method of liquefaction in food analysis; although a considerable amount of toxic acids are used. Our dry incineration has been improved so as to get carbon free ash more easily. A suitable standard range and dilution times for respective samples were checked and are shown in a table which can be easily referred to by readers. These are important for our student leaders in this experiment class, because those data can be easily used by each student to make respective experimental plan. Items in this experiment are described in succession.

Key Words: Quantitative analysis, Experimental practice, Food science, Inorganic ingredient

Subjects

1. Incineration procedure for mineral analysis

Air-dried cereals such as flour, germinating brown rice, polished rice, pressed barley and oatmeal were analyzed. About 10 g of each of the respective samples were placed in individually weighed previously ceramic

crucibles, and each crucible with its sample was accurately weighed. On the top of each sample in the crucible, 2 ml of 0.5% sodium sulfate solution was dropped as an incineration aid, and each sample crucible was transferred to an electric furnace kept at 90 °C until the samples parched. Then, the temperature of the electric furnace was raised to 460 °C, and the incineration (ashing) was continued until the next morning.

The ashing temperature of 460 °C is cited from a prior experiment in analyzing cadmium content in polished rice [1]. According to that condition, loss of cadmium, which has a lower melting temperature than zinc, was not found until a temperature of practically 500 °C was attained. In the present experiment, the furnace tempera-

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ture is set at a little lower, because samples and analyzing components differed from the prior case. In addition, it had also been found that some rice samples reacted with the glaze of the crucible producing black and barely soluble lumps which remained adhered to the bottom of the crucible when incinerated at 550 °C. Moreover, the temperature of the sample with sodium sulfate during incineration was elevated 50 °C more than the temperature of the sample without sodium sulfate. This furnace temperature is also favorable in preventing phosphorus evaporation or to the pyrophosphate formation during dry ashing [2].

2. Preparation of sample solution

About 1 ml of 1:3 diluted nitric acid was dropped around the inside wall of the crucible including the dark gray ashes to moisten the whole ash content in the crucible. The crucible with the nitric acid solution was transferred on a hot plate under a draft until the nitric acid fume disappeared. It was then transferred to the electric furnace set at 300 °C for around 20 minutes to promote the incineration. Whenever the ash includes some black particles, the above treatment with the nitric acid addition to the oxidation in the furnace at 300 °C should be repeated using a half ml or less of nitric acid. At the end of the nitric acid treatment, the inside ash of the crucible should show a purplish rose color, this color is the end mark of the ashing. Samples of brown rice and oatmeal usually required several repetitions of the nitric acid treatment, although the other samples could be oxidized rather easily.

The purple rosy ash was dissolved in 4 ml of 1:3 hydrochloric acid solutions, and the contents of the crucible were rinsed several times and poured into a 50 ml volumetric flask. When the volume reached about 90%, the contents of the flask were made uniform by shaking once. The contents were then adjusted to exactly 50 ml. This solution in the 50 ml volumetric flask was transferred into a 50 ml polyethylene bottle, and it was sealed against access of air, and preserved as the original sample solution.

3. Colorimetric determination of phosphorus by the vanado-molybdate method

The principle and notice on phosphorus analysis

The sample solution can be prepared by a wet digestion method or a dry incineration method; however, all phosphorus should be converted into orthophosphate form. This is essential, since the orthophosphate reacts with molybdate, and it becomes the phospho-molybdate,

and then, it combines with vanadate to form molybdo-vanado-phosphate [2].

In the course of the dry ashing at 460 °C, it is necessary that the incineration temperature remain below 500 °C. If it is not kept below 500 °C, there is a possibility that in materials containing abundant phosphorus, such as cereals, the phosphorus will volatilize at a temperature over 500 °C [2]. When black carbon granule remains after dry ashing, it is also said that pyrophosphate as well as orthophosphate may exist in the sample solution. In such cases, it is necessary to convert all phosphorus into orthophosphate by boiling the sample solution with a small quantity of nitric acid or by using the wet digestion method [2]. The dry ashing at 460 °C and succeeding nitric acid treatment in the present work are effective as a countermeasure to the above problems like phosphorus evaporation and pyro phosphate formation. When the color of the sample solution is yellow or red, this colorimetric analysis is not applicable, because the solution itself has absorption of around 410 nm.

Equipment, reagents and phosphorus standards

(1) A general photometer which can measure wavelengths neighboring 400 nm is a necessary instrument.

(2) The vanado-molybdate reagent

(a) 27 g of ammonium molybdate 4 hydrate $[(\text{NH}_4)_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}]$ are dissolved in 200 ml of hot water, and are then cooled.

(b) 1.12 g of ammonium metavanadate $[\text{NH}_4\text{VO}_3]$ are dissolved in 125 ml of hot water, after cooling, 250 ml of concentrated nitric acid are poured gradually on the ammonium metavanadate solution while mixing.

(c) The completion of the vanado-molybdate reagent

The solution (a) is gradually poured into the solution (b) which is gently whirling and cooling in a running tap water bath. The volume of the mixed solution is increased to 1 liter with the addition of pure water after the solution has cooled.

(3) The phosphorus standard undiluted solution

After 2 hours drying in an oven kept at 105 °C, and cooling in a desiccator, exactly 4.394 g of dried potassium dihydrogen phosphates are taken. The weighed phosphates are dissolved and diluted to exactly 1 liter using an addition of few ml of 0.05 N hydrochloric acid solutions. The accurate standard for this undiluted solution is 1 g phosphorus per liter.

(4) The daily used phosphorus standard

2, 5, 10, 15, 20 ml of the undiluted phosphorus

standards are poured into their respective 100 ml measuring flasks, and 0.05 N hydrochloric acid is added to attain a volume of 100 ml. The phosphorus quantities of those daily used standards are 2, 5, 10, 15, 20 mg phosphorus per 100 ml, respectively.

Procedure of coloration

5 ml of the newly prepared daily used phosphorus standards are put into respective 50 ml volumetric flasks. Each inside solution is carefully neutralized by several droppings of 1% (W/V) sodium hydroxide solution with a drop of alcoholic 1% phenolphthalein indicator. When a slightly pink color appears, the colorless condition is recovered by adding a few drops of 0.05 N hydrochloric acids. Then, the volume inside the measuring flask is increased to 35 ml or so by adding pure water. 10 ml of the vanado-molybdate reagent are poured and mixed, and the inside volume is adjusted exactly to the marked line of the volumetric flask by adding pure water, and finally it is mixed well.

The procedure of sample coloration is similar to that of the standards described above; however, the neutralization should be carried out prudently, since the quantity of acid differs with each sample, and the sampling volume of the original sample solution is changed, to adjust to a suitable phosphorus level. Usually the used volume of each original sample solution is adjusted to get 0.5 mg phosphorus level, which brings about 0.25 of absorbance.

According to the results of our preliminary experiment, the practical used volumes of the original sample solutions are 2 ml for the germinating brown rice and the oatmeal (25 times dilution) and 5 ml for the flour, the polished rice and the pressed barley (10 times dilution) as shown in Table 1. The cases of the other minerals are also given in Table 1. The colored flask is kept still for 30 minutes. Each absorbance is measured according to the ascending order of standard concentration after ample rinses with the the respective standard. The colored samples are measured in a similar way to the standards.

A calibration curve is drawn from the data corresponding to the respective standards. The data of samples are used to determine the phosphorus quantity contained in each sample referred to in the calibration curve. Then, the phosphorus quantity in the sample taken from the original sample solution is converted into the phosphorus quantity (mg) contained in whole 50 ml of the original sample solution. Referring to this phosphorus mg in the whole 50 ml of the original sample, the phosphorus content of 100 g of each sample food is calculated according to the accurate g weight of the air dried sample measured at the start. This content of phosphorus, mg in 100 g food, is directly comparable to that in the food composition table [3].

4. Quantitative determination of potassium by flame analysis

Heat energy is given to fine particles of the atomized

Table 1. List of daily used standard levels, dilution degree and wavelength in each element

○Phosphorus standards daily used	0	20	50	100	150	200 mg/l
Dilution degree for flour				10 times (5 ml/50 ml)		
rice and pressed barley				25 times (2 ml/50 ml)		
Those for germinating brown rice and pressed barley				Absorbance at 410 ml		
Measured wavelength						
○Potassium standards daily used	0	2	5	10 mg/l		
Dilution degree for all sample				100 times (1 ml/100 ml)		
Measured spectrum				K at 766.5 nm		
○Calcium standards daily used	0	10	20 mg/l			
Dilution degree for all sample				10 times (5 ml/50 ml)		
Measured spectrum				Ca at 422.7 nm		
○Magnesium standards daily used	0	2	5	10 mg/l		
Dilution degree for all sample				100 times (1 ml/100 ml)		
Measured spectrum				Mg at 285.2 nm		
○Iron and zinc standards daily used	0	2	5	10 mg/l		
Dilution degree for all sample				without dilution		
Measured spectrum				Fe at 248.3 nm Zn at 213.9 nm		

solutes; the given energy is converted to the potential energy of electrons which surround the atomic nucleus. The electrons having high potential energy are apt to go back toward the ground state emitting the specific spectra. The spectral energy which is emitted by the activated electrons can be used for analyzing each element. The specific emissions of alkali metals are of practical use in the flame photometric analysis, because the spectral emissions of such metals are stable, even when the given energy is low as in a gas flame. For our purpose, only sodium and potassium are important as objects of flame analysis.

In the present experiment, sodium sulfate is used as an ashing aide during incineration; therefore potassium is only the object of the flame analysis. About 0.3 mg per liter level of sodium (as sulfate) is added to the potassium standards to eliminate the matrix effect caused by a considerable amount of sodium. The above matrix negation procedure is thought to be useful to subtract the effect of impure potassium included in the sodium sulfate reagent. A yellow orange flame, due to the sodium sulfate, is present during the experiments, and the purple-red colored potassium flame is invisible to the naked eye. The spectroscopy in the inside of the flame analyzer system works to isolate the energy of potassium spectra from the sodium rich flame, and the recorded output only draws potassium intensity.

Preparation of potassium standard and sample solution

Potassium chloride is dried at 102 °C for 2 hours, and is cooled in a desiccator. 1.907 g of dried potassium chloride is weighed and dissolved in about 50 ml of 0.05 N hydrochloric acid solution, and it makes 1 liter in a volumetric flask. The strength of the stock standard solution is 1.000 g of potassium per liter (1000 mg/l). As shown in table 1, the dilution degree of the analyzing sample solution for potassium was 100 times, and the suitable potassium standard range was from 0 to 20 mg/l as shown in table 1. These potassium standards were prepared easily using 0, 2, 5, 10 and 20 ml of the 10 times diluted stock standard per 100 ml.

The potassium spectrum at 766.5 nm of wavelength is used for the present flame analysis, and 100 times dilution of the original sample solution is sprayed. In the present experiment, sodium sulfate was added as an ashing promoter which is thought to be a target of the matrix effect. On the other hand, this means one of regular matrix correctors is added already, because phosphate or

sulfate is used frequently as a corrector of matrix effect. As a consequence, some amounts of sodium sulfate give a considerable sodium emission to the burner flame. However, the potassium spectrum can be isolated and eliminated from the sodium spectrum by the spectroscopy in the flame photometer.

Sample liquid is sprayed and mixed into the atomizer burner of the photoelectric flame photometer, and the intensity of the potassium spectrum is measured and recorded. Finally, the potassium quantity which exists in the original sample solution is converted to be mg of potassium contained in 100 g naturally dried sample which is directly comparable with the data in the food composition table in Japan [3].

5. Quantitative determination of calcium and magnesium by atomic absorption spectrometry

From 1950's to 1970's, the flame analysis had become popular and was used widely in Japan as it had brought about accurate results, especially in the analysis of alkali metals such as sodium and potassium. As a result of the spectroscopic analysis of solar light, the light energy which corresponds to the bright line spectra of sodium is absent. The idea of the atomic absorption spectrometry is a result of that phenomenon, and a new analyzer system for atomic absorption spectroscopy was devised.

The optical pathway or flow of the signal of the atomic absorption spectroscopy is as follows: From the hollow cathode lamp which emits the specific spectra of each element and the atomizer-burner where the bright line energy from the hollow cathode lamp is absorbed according to the quantity of corresponding element included in the given sample, to the spectroscopy part, where the objective spectrum is isolated from many other unrelated spectra, and finally, to the photomultiplier which is the highest sensitive photoreceptor get a corresponding signal, besides the signal recorder draws the absorbance signal after the initial transmittance signal was converted to the absorbance base.

Up to the present, the spectroscopy has undergone many technical developments and improvements, for example, from a glass prism to a rock crystal prism and a diffraction grating which gives uniform dispersion of spectra from a red zone to an ultraviolet zone. While the atomic absorption spectrometric apparatus at this university is old-fashioned, it has a combined system of the diffraction grating, the photomultiplier and the signal converter. Therefore, good functions can be utilized immediately and conveniently at present.

The atomic absorption spectroscopic system in our university can also be utilized for the flame analysis of calcium and magnesium; however, matrix effects in flame analyses of those elements are severe. Meanwhile, the atomic absorption spectrometry can be easily applied to several popular elements such as iron, manganese, potassium, sodium zinc and so on.

Gradations of standard concentrations of calcium are established at levels of 10, 20 and 30 mg per liter as shown in table 1, because sensitivity for calcium is a little low at the measurement wavelength 422.7 nm, and the sample solution prepared may not contain so much calcium. Magnesium is measured at 285.2 nm. Magnesium standards are determined at 0, 2, 5 and 10 mg per liter levels because of its high sensitivity and stable output. The spectrum from each hollow cathode lamp was monitored, and each absorbance which was detected in each supply of sample or standard of each element was recorded. The above process in the atomic absorption analysis was the same in the case of the other elements. Besides, 10 times dilutions from the original sample solution were adapted to the calcium analysis, and 100 times dilutions to magnesium as shown in table 1.

The calibration curve for each element was drawn using corresponding outputs of respective standard concentrations. Concentrations of calcium and magnesium contained in respective analyzing sample solution are calculated by the comparison between outputs of samples and standards on each calibration curve. Resulted concentrations of both minerals are converted finally to the comparable level of content in food sample to the data of the food composition table in Japan [3].

6. The Quantitative determination of iron and zinc by atomic absorption spectrometry.

The equipment of the atomic absorption spectro-

photometer was used for the determination of iron and zinc too. In general, a level of sample dilution is selected within limits where the linear increase of output can be found, but is preferably not less than the lowest standard. The output of the sample is less than the upper limit; the concentration of the sample dilution can be calculated by a linear function. If the standard 0 shows little output, for example, by a reagent addition to overcome matrix effect, the output of 0 must be subtracted from each output. When the sample output was more than the linear increasing area, re-analysis using more diluted sample was necessary, but an excess of output was minimal and the error caused by the excess output was estimated to be only a very small percentage. Temporary data can be calculated provisionally. As shown in table 1, the original sample solution was supplied directly to the atomic absorption spectrophotometer in the case of both iron and zinc. Therefore, each concentration in the sample solution can be easily found on each calibration curve. From iron and zinc concentrations in the original sample solution, respective mg contents in 100 g air dried foods are calculated.

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