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Rapid Measurement of Phytate in Soy Products by Mid-infrared Spectroscopy

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ABSTRACT: The rapid measurement of phytate from soybean and various its product such as soy flour, defatted meal, soymilk and tofu was investigated using Fourier transfer infrared spectroscopy (FT-IR) with an ATR accessory. The phytate, separated from protein by trichloroacetic acid (TCA), was precipitate completely by the addition of calcium on pH>7.0 even in the presence of TCA. The precipitate was dissolved in citrate buffer (pH 6.0) and then used for IR measurement. The absorbance at 1070cm-1 correlated well the phytate content of the each sample. The measurement of phytate can be done rapidly by FT-IR with an ATR accessory and gives reproducible values.

Key words: soy product, phytate, infrared spectroscopy, FT-IR

Introduction

Soybean foods such as soymilk, tofu (soybean curd), and its derivatives have been popular in some Asian countries since ancient times. Their consumption today has been spread gradually all over the world as healthy foods. Defatted soy meal (residue of soybean oil) and its products such as soy protein isolate were important sources of protein for human and livestock.

Soybean contains 1-3% phytate that is common components of grains and legumes. Since phytate can form insoluble complexes with minerals, reducing the availability of them (Sandberg and others 1991; Mark and others 2000), it is usually regarded as an antinutrient. On the other hand, recent research indicates that it has beneficial roles as an antioxidant and anticarcinogen (Minihane and others 2002). Phytate is also known to affect to tofu curdling (Saio and others 1969). Because the quality of tofu depends mainly on its physical properties, phytate is important on tofu making process.

Many methods for phytate measurement have been investigated. Some methods for measurement using ferric precipitation (Makower 1970), HPLC (Kunckles and others 1982), or other methods (Kayama and others 1998) are established, but these are too time-consuming to use on manufacture for a large number of soybean or its products.

Recent research has shown that Fourier transfer infrared spectroscopy (FT-IR) is a simple, rapid, and reliable method to measure some food components. Phytate in raw soymilk has been reported to be able to measure by using IR method. (Ishiguro and others 2003) Since the method is not able to apply to other soy products, we investigated an IR analysis method for the phytate measurement of various soy products such as raw soymilk, soymilk, okara (extract residues), and tofu on tofu processing, and

soy meal, soy additives, soy drinks and others.

Materials and Methods

Materials

The soybean (species Glycine max var. Suzuyutaka) was harvested at the Iwate University Experimental Farm located in Morioka, Iwate, Japan, and was stored at 4℃ until use.

0.5 M citrate buffer was prepared by 0.90 g of citric acid monohydrate and 13.44 g of trisodium citrate dihydrate dissolving in 100 ml of water. The pH of the solution was about 6.0.

Preparation of soy flour, defatted soy meal, and whole bean soymilks

Soy flour was prepared from whole soybean by milling with food-mill (Model FM-50, San Ltd., Tokyo, Japan) until flour pass through the sieve of 450 μm. Lipid of soy flour was extracted by n-hexane, and the residue was designed defatted soy meal.

Soy flour (5.0 g) was mixed into a homogenate with 50 ml of water using an Oster blender (Oster Co., Milwaukee, Wisc.), and the homogenate was designed as whole bean soymilk. Defatted soy meal (5.0 g) was mixed into a homogenate with 50 ml of water using an Oster blender and the homogenate was designed as defatted whole bean soymilk.

Preparation of raw soymilk, okara, soymilk and tofu

The soybean seed (10.0 g) was soaked in deionized water for 18 h at 4℃. The swollen beans were ground into a homogenate with 70 ml of water using an Oster blender and the homogenate was then filtered through a defatted cotton sheet. The residue was designated as okara. The filtrate was designated as raw soymilk. Soymilk was prepared by heating the raw soymilk in a boiling bath for 5 min above 95℃. Tofu was prepared from the

soymilk by adding 0.3% GDL and following in water bath for 60 min at 90℃. The weight of each product was recorded.

Preparation of soy saccharide and protein solutions

Sucrose (0.25 g) and stachyose (0.25g) were mixed and dissolved in 10 ml of water for soy saccharide solution. Soy protein was prepared by the method of Thanh and others (1975). Its solution was made up to 3% for the soy protein solution.

Preparation of standard phytate solution for IR method

0.137 g of calcium phytate was dissolved in 10.0 ml of 0.5 M citrate buffer (pH6.0) (1.00% solution as phytic acid), and the solutions of various concentrations were prepared by diluting with 0.5 M citrate buffer. The absorbance of the citrate buffer without phytate was used for the blank value of these standard measurements.

Measurement of precipitated phytate by addition of calcium chloride

To measure the calcium need to precipitate phytate completely, various concentrations of calcium were added to phytate solutions. And then the precipitated phytate was measured by IR absorbance.

Each 0.5 ml of Potassium phytate solution (about 1% as phytic acid: exact concentration for this solution was determined by the method of Makower (1970)) was stood for 20 min after mixing with 0.5 ml of 12% TCA, and 0.5 ml of 1 N NaOH was added for neutralization. Various volumes (0 to 0.10 ml) of 1 M $CaCl_2$ were then added to each of this phytate solution. The mixture was settled at room temperature for 10 min, and then centrifuged at 8000 x g for 3 min. The precipitate containing phytate was separated and drained. The precipitate was then dissolved in

0.5 ml of 0.5 M citrate buffer. The phytate concentration was measured by infrared (IR) spectra measurement and converted to a percentage from the presumed value that all phytate was precipitated. The values were obtained for the average of five times, and standard deviation was calculated.

Separation of phytate from each sample

All samples were deproteinized at first by addition of trichloroacetic acid (TCA). In the case of solid matter (soy flour, defatted meal, okara and tofu), 0.1-0.3 g of each sample was mixed with 1.0 ml of 6% TCA. In the case of liquid matter (raw soymilk and soymilk), 1.0ml of sample was mixed with 0.5 ml of 18% TCA. The mixture was stood at room temperature for 20 min, and then centrifuged at 8000 x g for 3 min. One ml of the deproteinized supernatant was mixed with 0.5 ml of 1 N NaOH and 50 μ l of 1 M $CaCl_2$. The mixture was stood at room temperature for 10 min, and then centrifuged at 8000 x g for 3 min. The precipitate containing phytate was separated and drained. The precipitate was then dissolved in 0.5 ml of 0.5 M citrate buffer. This solution was used as the sample for infrared (IR) spectra measurement of phytate. The phytate precipitate must be dissolved for IR measurement with ATR apparatus. A dissolving solvent having buffer effect is needed to prevent any variations of IR spectra with pH change. It also requires that IR absorption of the solvent is as little as possible in the region of phytate. Therefore, 0.5 M sodium citrate (pH 6.0) was used for this purpose. In fact, the buffer had IR absorption about 0.04 in the region of phytate. However, it was no problem because the affect was able to remove by subtraction of blank from each data.

Measurements of IR spectra

IR spectra of raw soymilk, soy components, and phytate solution were

measured from 4000 cm^{-1} to 800 cm^{-1} with a FT-IR spectrophotometer (SPECTRUM2000, Perkin-Elmer Ltd, Beaconsfield, England). An overhead-attenuated total refraction (ATR) accessory (the horizontal-ATR 45° ZnSe crystal cell, Spectra-Tech Inc., CT) was equipped as the sample stage for liquid samples. All spectral measurements were done at 4 cm^{-1} resolutions. The single-beam ATR spectrum from each sample was corrected using a background spectrum of deionized water, and transformed to absorbance units. The values were obtained for the average of five times measurements.

Result and Discussion

IR spectra of phytate and major soy components

Soybean generally contains 1-3% phytate, and soymilk contains 0.1-0.4% phytate. Whole soybean is a source of all soy products, and many types of tofu and drinks are derived from soymilk. We tried to measure these phytate contents by IR absorption spectrophotometry.

The IR spectrum of 1% potassium phytate solution was obtained by using a FT-IR spectrophotometer equipped with an ATR apparatus and is shown in Figure 1. The IR spectrum of phytate in 1200-900cm^{-1} region had four peaks, which were at 970cm^{-1}, 1070cm^{-1}, 1124cm^{-1}, and 1170cm^{-1}, being due to -PO_3, C-O-P, C-C, and P=O, respectively.

IR spectra of raw soymilk and its components (saccharide and protein) were then measured and are shown in Figure 2. Soy saccharide had absorption in 1200-900cm^{-1}, and soy protein also had a small absorption in same region. These results make it difficult to measure the phytate content in soy products directly by IR measurement. The phytate content could be measured in principle by subtracting these absorbencies. However, the phytate content of the samples is minute as compared with those of saccharide and protein, so that the subtracted values for phytate would include large errors.

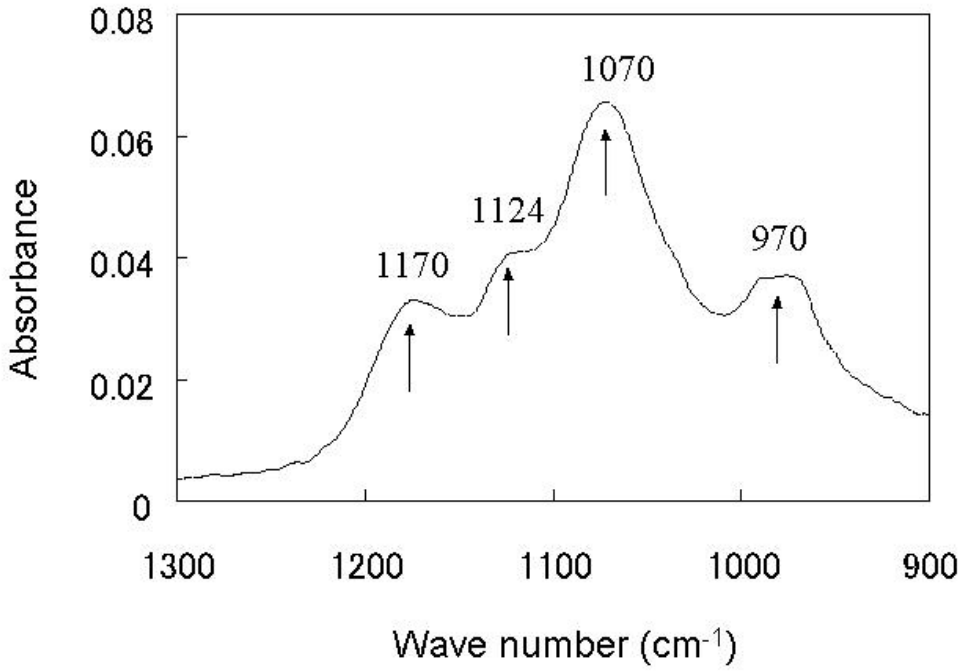

Figure 1-IR Spectrum of potassium phytate in aqueous solution by FT-IR measurement with ATR apparatus. The blank was water.

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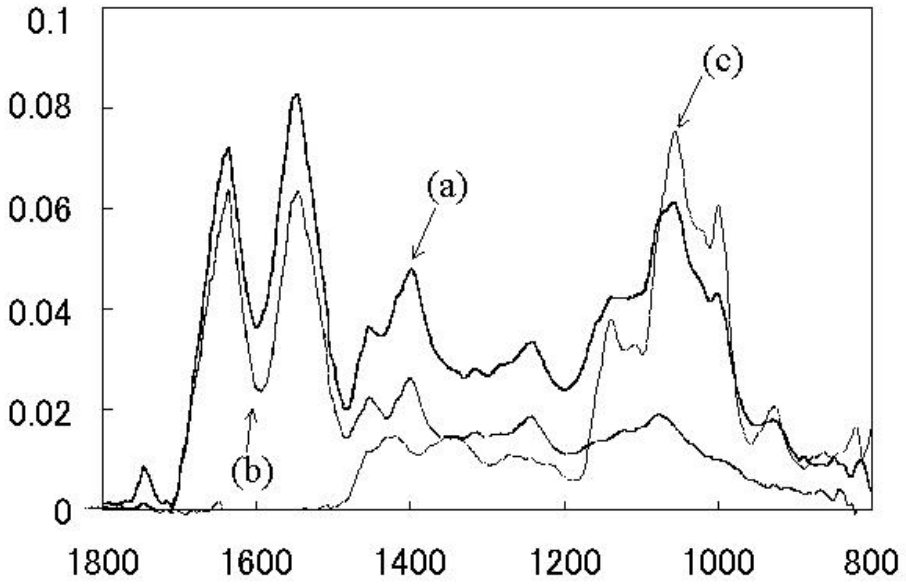

Figure 2-IR Spectra of raw soymilk (a), soy protein (b), soy saccharide (c) in aqueous solution by FT-IR measurement with ATR apparatus. The blank was water.

Separation of phytate by addition of calcium

Separation of phytate from samples was required for accurate IR measurement with ATR apparatus. However, a separation of phytate by conventional methods such as HPLC takes much time (Kamaya and others (1998) reported that the extraction and separation of phytate was needed for 5 hours). The rapid procedure, therefore, precipitating of phytate by calcium was used. It is shown that phytic acid is soluble in water, but precipitates by calcium ion at above pH 5.0 (Lasztity 1990). If the solution contained proteins, phytate was not precipitated completely (Ishiguro and others 2003). Therefore, the removing protein from samples was done by the addition of TCA. We tried to use 3 and 6 % of TCA for each sample. 3 % was not enough to precipitate proteins in soy flour homogenate but 6 % was completely. Other products (raw soymilk, soymilk and tofu) were able to use both of 3 and 6 %. In this experiment, 6% TCA was used for the removing protein from samples. After removing protein, the solution was neutralized since calcium phytate does not precipitate below pH 5.

Calcium was added gradually to potassium phytate solution containing neutralized 6% TCA. Phytate was precipitated completely by the addition of calcium more than 6-times (mole) of phytate as shown in Figure 3.

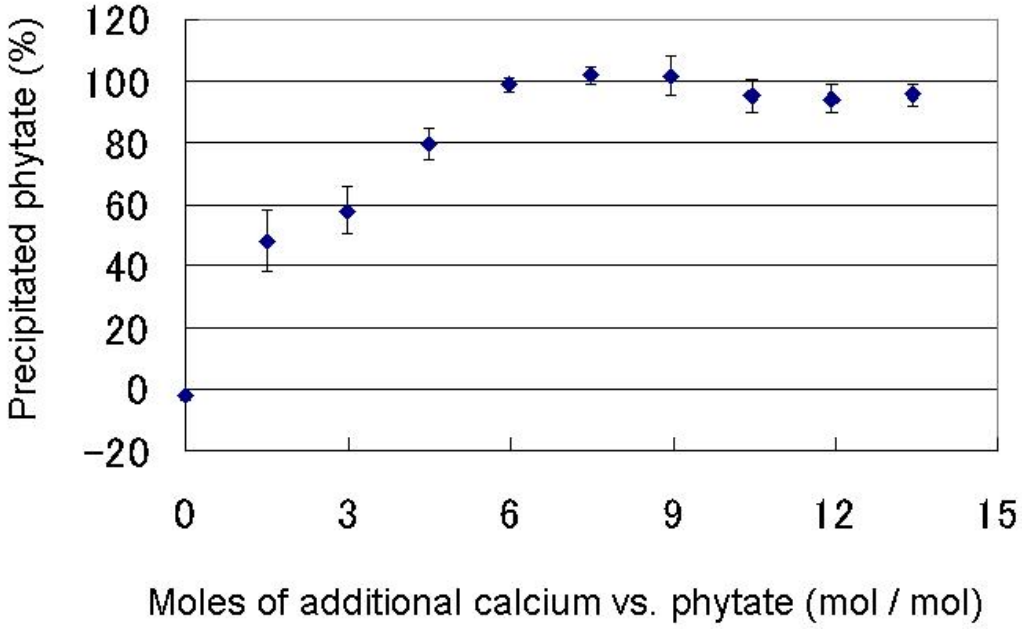

Figure 3-The ratio (%) of precipitated phytate from potassium phytate solution containing neutralized 6% TCA by the addition of calcium chloride.

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Standard calibration of phytate in citrate buffer by IR method

A standard solution was obtained from calcium phytate dissolved in citrate buffer. The IR spectrum of calcium phytate was already shown in figure 1. The citrate buffer was used for blank. The spectrum showed the maximum absorbance at 1070 cm^{-1}. Figure 4 shows absorbencies of 1070 cm^{-1} from various phytate concentrations. Their absorbance and concentration were strongly correlated (coefficient of measurement >0.99), suggesting this IR method is available for the measurement of phytate content. The absorbance at 1070 cm^{-1} and this standard calibration were used for the measurement of phytate content by IR method.

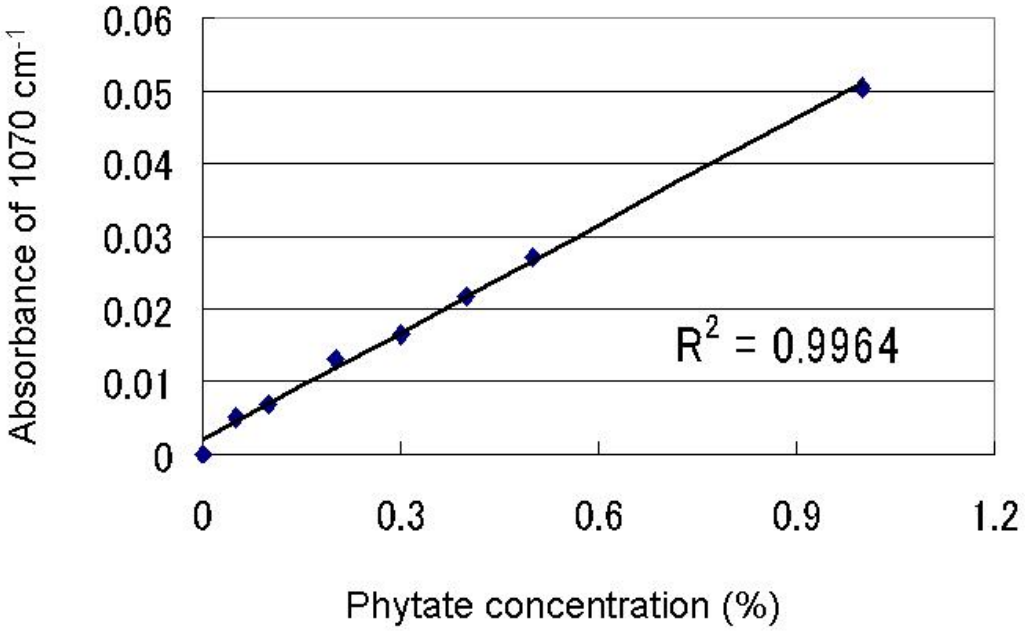

Figure 4-Standard calibration of phytate obtained from calcium phytate dissolved in citrate buffer. The blank was citrate buffer.

Phytate contents of soy flour, defatted meal, and whole soymilks

Whole soybean is a source of all soy products, and plenty of defatted soy meal is used for food and feedstuff. Phytate contents of them were measured by the IR method, and the determined values were compared with those by the conventional method (Makower 1970) as shown in Table 1. The values were obtained for the average of five times, and standard deviation was calculated.

Phytate contents of Soy flour by IR and conventional methods were 2.14 ± 0.14 % and 2.01 ± 0.32 %, respectively. Then those of defatted soy meal were 3.00 ± 0.12 % and 3.11 ± 0.22 %, respectively. This result indicates the values by the IR method is reliable and capable of greater precision than that by conventional method, because two values obtained by two methods were almost same and the value by IR has smaller standard deviation than that by conventional method.

Since dietary fiber has effective bioactivity, much attention is being denoted to whole bean soymilk. The whole bean soymilk was made from soy flour by mixing into a homogenate with 10 times water of its weight, and defatted soy meal were prepared by defat the soy flour. These phytate contents were measured by the IR method. The phytate content of whole bean soymilk were 0.22 ± 0.03 %, which was just one tenth of the source (soy flour). The value of defatted soy meal was 0.28 ± 0.02 %, which is consistent with the content in the material subtracting lipid (21.5%) from soy flour. The IR method, therefore, can be applicable to whole bean soymilk and defatted soy meal, too.

Table 1- Phytate contents of soy flour and defatted soy meal determined by IR and conventional methods.

	IR method (%)	The method of Makower (%)
Soy flour	2.14±0.15[a]	2.01±0.32
Defatted soy meal	3.00±0.12	3.11±0.22
Whole bean soymilk	0.22±0.03	ND[b]
Defatted whole bean soymilk	0.28±0.02	ND

[a] Data are the means of 3 replicate ± standerd deviation

[b] Not determined

Phytate contents of products on tofu making process

Tofu is made by soymilk curdling, which process is affected by phytate (Saio and others 1969). So phytate contents of materials on processing should be pay attention in tofu making. And then, appreciation of the IR method to these materials was investigated.

Soy products (okara, raw soymilk, soymilk and tofu) were prepared in a tofu making process and these phytate contents were measured by the IR method. The results are shown in Table 2. The values were obtained for the average of five times, and standard deviation was calculated.

Since phytate content of the soybean was 2.14 %, 10.0 g soybean contains 0.214 g phytate. Sum amount of phytate in raw soymilk and okara should be agreed with that of source soybean. Raw soymilk and okara were made from swollen soybean and water. Yield of raw soymilk and okara was 90 % on this experimental scale. Phytate contents of raw soymilk and okara were 0.242 % and 0.170 %, so amount of phytate in each sample were 0.157 g, and 0.029 g, receptivity. Sum of them were 0.186 g, so that 87 % of phytate in soybean was recovered in raw soymilk and okara. Since overall weight yield was 90 %, phytate content could be regarded reasonable experimental values.

Soymilk made from raw soymilk by heat is a beverage itself and is just a source of tofu. Phytate content of the soymilk was 0.252 ± 0.015 %, almost same with raw soymilk. Phytate content of tofu was 0.230 ± 0.018 %, almost same with soymilk and raw soymilk. Those results indicate the IR method can be a capable measurement of phytate content in soymilk and tofu as well as soy flour and raw soymilk.

Table 2- Phytate contents of tofu and its intermediate products measured by IR method.

	weight of products(g)	Phytate concentration(%)	Total amount of phytate(g)
Soy bean	10.0	2.14±0.15	0.214
Swollen bean and water	91.2(100)[a]	-	0.214(100)
Okara(residue)	16.9(19)	0.170±0.035	0.029(13)
Raw soymilk	65.0(71)	0.242±0.012	0.157(74)
Okara and raw soymilk	81.9(90)	-	0.186(87)
Soymilk	64.9(71)	0.252±0.015	0.163(77)
Tofu	65.0(71)	0.230±0.018	0.149(70)

[a] Numbers in parentheses represent the recovery against source.

Limitation on measurement

When the phytate was less than 1 μmol (= 0.7 mg/L) in sample, it was difficult to collect calcium phytate as a precipitate from a sample. On the other hand, when the large amount of phytate is in sample, it won't be precipitate completely because of insufficiency of calcium. Amount of calcium was needed more than 6 times of phytate for complete precipitation. Appropriate amount of samples and additional calcium should be used. The phytate content of soybean is almost in 1 - 4%. Therefore, the phytate contents in soybean and its products can be measured accurately by this IR method.

The other inositol phosphates (inositol 1～5 phosphates) may disturb the measurement of phytate. Though these types are scarce in native soybeans (Sandberg and Ahderinne 1986), fermented soy foods such as miso and natto may contain much more (Ohtsuki and others 2001).

Conclusion

Phytate was precipitated by calcium from deproteinated soy products. Precipitation containing phytate was dissolved in citrate buffer, and then measured by FT-IR with ATR apparatus. Absorbance of 1070cm^{-1} was correlated to phytate concentration well.

This IR method could be measuring various form of phytate such as in soy flour, soymilk and tofu exactly. This result shows it will be used not only soy products but also other foods or feeds for phytate measurement.

The method of Makower (1970) has been used for many experiments, but is a very complex procedure and needs much time. Our method by IR measurement can do by using only a little of samples and takes less than 50 min for the separation of phytate and analysis of IR. Phytate measurement by the IR method is a more simple and rapid method than the conventional

ones.

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