Food Sci. Technol. Res., **10** (2), 137–142, 2004

Rapid Quantitative Analysis of the Major Components in Soymilk Using Fourier-Transform Infrared Spectroscopy (FT-IR)

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Received July 14, 2003; Accepted December 16, 2003

Fourier-transform infrared spectroscopy (FT-IR) on attenuated total reflectance (ATR) sampling was used for the quantitative analysis of the major components (protein, lipid, and sugar) in soymilk. Since mid-infrared spectroscopy shows specific absorption of each functional group of each molecule, it is possible to determine the amount of each component without complicated statistical computation. The determination of protein content was performed by using amide II absorbance at wavenumber 1545 cm^{-1} for protein, ester absorbance at 1745 cm^{-1} for lipid, and C–C·C–O absorbance at 1000 cm^{-1} for sugar. The protein and lipid contents were determined directly from these absorbencies, and the sugar content was calculated by subtracting the effect of protein. There was a linear relation ($R^2 > 0.99$) between each absorbance and each concentration of purified components (soybean protein, lipid, or sugar), and the values by this method were well consistent with those by chemical method. This technique of measurement of the major components in soymilk by FT-IR with ATR is more convenient and rapid.

Keywords: FT-IR, ATR, soymilk

Soybean foods, such as soymilk and tofu, are popular in some Asian countries. The quality of tofu, which is manufactured by curdling soymilk with coagulant (magnesium chloride and so on), depends largely on the content of the major components (protein, lipid, and sugar) in soymilk. It is known that these concentrations are influenced by the difference of soybean species and of the soymilk manufacturing process and environment. Conventional analyses, that is, the Kjeldahl and other chemical methods for protein content, the Soxhlet method (extracts by ether) for lipid content, etc. require complicated and time-consuming chemical analyses.

Infrared spectroscopy (IR) is used to determine molecular structure. However, it is possible to get quantitative infrared spectrum by incorporating the Fourier transform (FT) method. Furthermore, recently FT-IR has been widely used for quantitative analysis of components in some foods by improved statistical analysis (partial least squares (PLS) regression, etc.). While, attenuated total reflectance (ATR) is suitable for analysis of filmy and liquid samples for which KBr-disk analysis is difficult. Since ATR was invented, MIR on ATR has been used for analysis of components in vegetable oil (Lai *et al.*, 1994), instant coffee (Briandet *et al.*, 1996), fruit purees (Defernez *et al.*, 1995; Kemsley *et al.*, 1996), and meat products (Jowder *et al.*, 1999). There are various applied examples like the discrimination of red wine cultivars by phenolic wine extracts (Edelmann *et al.*, 2001) and the determination of ammoniacal nitrogen in farmyard manure (Kemsley *et al.*, 2001). These are simple and rapid methods for quantitative analysis of components in foods. Since the analysis at Mid-infrared shows specific absorption of each functional group of the molecule, it is possible to perform quantitative analysis of major components without complicated statistical computation. We report here the rapid and simple analysis of the major components (protein, lipid, and sugar) in soymilk using FT-IR on ATR sampling.

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Abbreviations: FT-IR, Fourier transfer infrared spectroscopy; ATR, attenuated total refraction.

Materials and Methods

Materials A main sample (species *Glycine max* var. Suzuyutaka) of soybeans was harvested at the Iwate University Experimental Farm located in Morioka, Iwate, Japan, and was stored at 4°C until use. Other soybeans were obtained from various districts in Japan as shown in Table 1.

Preparation of soymilk Soybeans were soaked in deionized water for 18 h at 4°C. The swollen beans were ground into a homogenate with 8 times deionized water of the soybean weight using an Oster blender (Oster Co, Milwaukee, USA). The homogenate was filtered through a defatted cotton sheet, and the filtrate was designated as raw soymilk containing 8%(w/w) solids. The raw soymilk heated in boiling water for 5 min was designated as soymilk containing 8% solids.

Instrumentation and spectral acquisition FT-IR spectra were measured by a Fourier transfer IR spectrophotometer (SPECTRUM2000, Perkin-Elmer, Ltd, Beaconsfield, England) equipped with a horizontal-ATR 45° ZnSe crystal cell (miller angle 45°). Measurement of a sample of soymilk is simple; soymilk 1 ml was placed on the ZnSe crystal cell. The IR region measured was between 4000 and 800 cm^{-1} with 4 cm^{-1} resolution. This is the standard measurement condition for this

Table 1. Soybeans, used in this study.

Cultivar name	Year harvested	Place
Suzuyutaka	1999	Iwate
Tamahomare	1999	Hiroshima
Ichihime	1999	Hiroshima
Enrei	1999	Hiroshima
Ohsuzu	1999	Akita
Tachikogane	2000	Iwate
Kariyutaka	2000	Hokkaido
Nanbushirome	1999	Iwate
Toyohomare	2000	Hokkaido
Ryuhou	1999	Akita
Tachiyutaka	1999	Akita
Toyokomachi	2000	Hokkaido
Tomoyutaka	1999	Akita
Fukuyutaka	1999	Fukuoka

instrument. Single-beam ATR spectrum from each sample was corrected using the background spectrum of water contained in the sample and transformed to the absorbance unit. The ratio of water in sample was calculated from the absorbencies between 2300 and 1900 cm^{-1} because soymilk has no absorbance in this region. The decreasing rate of water content in sample was obtained by subtracting absorbencies of sample between 2300 and 1900 cm^{-1} from those of water (1 cm^{-1} interval) and averaging these values. The background spectrum of water for that of sample was obtained by considering this decreasing rate. There were 5 scans, and the spectral data were obtained from averaging the 5 scans. The measurements were repeated three times, and the absorbance data were obtained by averaging 3 values.

Preparation of soy-protein, soy-lipid, and soy-sugar Soy-protein was prepared from hexane-defatted soybean meal by the method of Thanh *et al.* (1975), dialyzed at pH8.0, and freeze-dried. It was dissolved in 50 mM KCl solution for soy-protein solution. Soy-protein solution was used to measure IR-spectra of soy-protein. 50 mM KCl solution was used as the mineral solution of soymilk.

Soy-lipid was prepared as reconstituted oil-body formed by adding soybean oil (Nacalai Tesque, Inc.) to defatted soymilk and then doing ultrasonic treatment. Hexane was used to defat soybean meal because defatting with hexane did not influence the size-distribution of protein particles in soymilk and the protein components from the hexane-defatted soybean meal were similar to those from whole meal (Ono *et al.*, 1996). Defatted soymilk was prepared from hexane-defatted soybean meal by the same method as used for soymilk. Soybean oil was added to the defatted soymilk and ultrasonic treatment was done with an ultrasonic disruptor (UD-201, TOMY SEIKO Co.) for 5 min because reconstituted oil-body was formed by this treatment (Tzen & Huang, 1992). The soybean oil content was adjusted from zero to 4% (w/w) including general soymilk. This reconstituted oil-body was designated as soy-lipid in this experiment. IR-spectrum of soy-lipid was obtained by subtracting IR-spectrum of the defatted soymilk from that of reconstituted oil-body soymilk.

Soy-sugar was prepared as a saccharide mixture with equal weights of sucrose (Kanto Chemical Co., Inc.) and stachyose (Sigma Chemical Co.). It was dissolved in 50 mM KCl solution for soymilk sugar solution, and this solution was used to measure IR-spectra of soy-sugar.

Chemical analysis The protein content of each soymilk was measured by the method of Bradford (1976) after defatting treatment revised by Tezuka *et al.* (2000). A good relationship of protein concentrations and measured values was reported in soymilk.

The lipid content was measured by the Soxhlet method. A sample for the determination was prepared by freeze-drying, and then was extracted with ether. The extract was dried, and weighed to determine its lipid components.

The sugar (sucrose and oligosaccharide) content was measured by subtracting ash-weight from the weight of de-lipid, de-protein, and freeze-dried sample. The de-lipidizing soymilk was done by centrifugally removing the floating fraction at 156,500×*g* for 30 min at 20°C. The de-proteining was performed by ultrafiltration. The ultrafiltrate of de-fatted and de-proteined soymilk was prepared by an ultrafiltration with YM-10 membrane (molecular weight cut off=10,000; Millipore Co.), and was then freeze-dried. Ash was obtained from the freeze-dried sample with an electric furnace at 550°C for 10 h.

Results and Discussion

IR-spectrum of soymilk IR-spectra of soymilk had several absorption regions as shown in Fig.1. The absorption regions of peaks at 2925 and 2855 cm^{-1} are attributed to CH_2 and CH stretching vibration bands, at 1745 cm^{-1} to carbonyl group of triglyceride ester, at 1640 cm^{-1} to amide I band, at

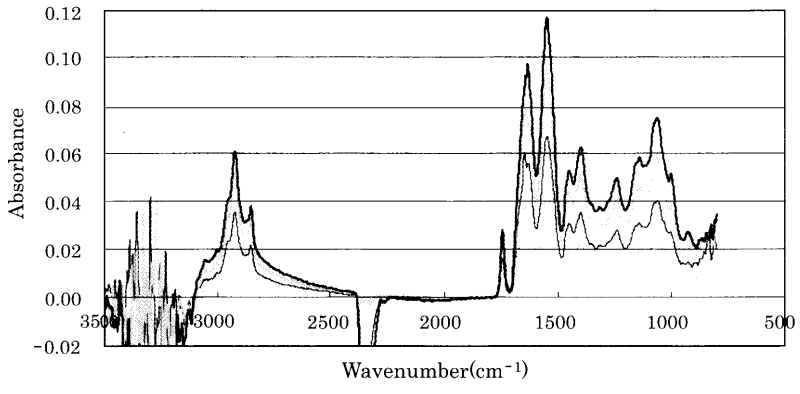

Fig. 1. IR spectra of soymilk in various solid contents. IR spectra of soymilk were measured using a FT-IR spectrophotometer equipped with an ATR apparatus. Order of IR spectra from the top is soymilks prepared with 6, 8, 12, and 20 times de-ionized water of the soybean weight.

1545 cm^{-1} to amide II band, and the region between 1200 and 900 cm^{-1} to C–C and C–O stretching modes. As all samples are aqueous solution, the region near 3300 cm^{-1} and 1600 cm^{-1} due to strong absorption of water molecule is noisy in all spectra. The absorption region near 2350 cm^{-1} is due to CO_2 gas during light path. When the solid content in soymilk was changed, there was a linear relation (R^2>0.99) between the absorbance and the solid content on all absorption bands (excluding the regions near 3300 cm^{-1}, 2350 cm^{-1} and 1600 cm^{-1}). Therefore, it is possible to measure the contents of major components (protein, lipid, and sugar) in soymilk by the absorbance of mid-IR spectrum.

In general, the mid-IR spectrum of aqueous solution must be measured considering water content, because water has strong absorption in the mid-IR region. When solid contents of soymilk were changed from 3% to 12%, the difference spectra of water were obtained from the change in the absorbance between 2300 and 1900 cm^{-1} because soymilk has no absorbance in this region. Difference in the IR-spectrum of water at 10% solid content is shown in Fig. 2. This Δabs was obtained by applying IR spectrum of water and the average ratio of absorbance changes of soymilk (10% solid) between 2300 and 1900 cm^{-1}. The correction of soymilk spectra by Δabs of water was below 5% (=Δabs/absorbance of sample) at 10% solid.

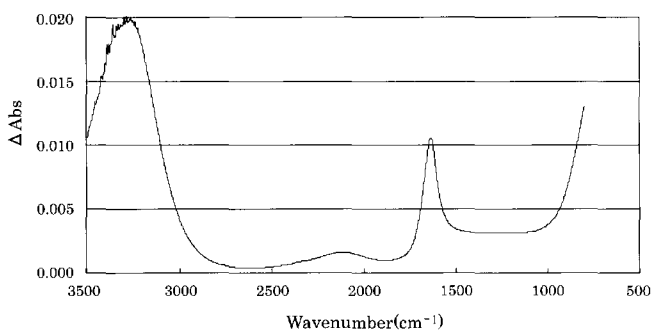

Fig. 2. IR spectra of water (using a background spectrum of air). ΔAbs of the vertical axis was obtained by applying IR spectrum of water and the average ratio of absorbance changes by soymilk of 10% solid between 2300 and 1900 cm^{-1} (that is the small and broad absorption band of water although soymilk components have no absorption).

Therefore, the absorbance values of soymilk were scarcely changed with or without the Δabs correction except for amide I absorbance near 1600 cm^{-1}. When the water spectrum was used for background measurement, the absorbance of soymilk spectrum was proportional (R^2>0.99) to the solid content (under about 10%) in soymilk (excluding the regions near 3300 cm^{-1}, 2350 cm^{-1} and 1600 cm^{-1}). Therefore, the concentration of water in a sample can be regarded as constant. However, for more rigidity, all spectra in this experiment were corrected considering the water contained in a sample.

In these measurements on the same sample, a difference of absorbance of less than 0.00 1 could be detected (excluding the regions near 3300 cm^{-1}, 2350 cm^{-1} and 1600 cm^{-1}). Consequently, the contents of protein, lipid, and sugar in soymilk can be detected in 0.01% viable order. This measurement precision deteriorated at less than 0.01 at absorbance. Therefore, the content of the major components in soymilk using FT-IR can be measured accurately at more than 0.1%.

IR-spectra of soy-protein, soy-lipid, and soy-sugar IR-spectra of soy-protein (soybean protein isolate), soy-lipid (oil-body), and soy-sugar (sucrose and oligosaccharide) in aqueous solution are shown in Fig. 3.

Amide I band (peak at 1640 cm^{-1}) and amide II (peak at 1545 cm^{-1}) band remarkably appeared in the spectrum of soy-protein solution. The absorption regions of amide I and II are attributed to protein, and not to lipid and sugar. If amide compounds are contained in soymilk, the amide absorbencies should contain these compounds, but the ultrafiltrate of soymilk had no amide absorbance. Furthermore, absorbance of these bands had a high correlation with protein content. However, soy-protein of soybean protein isolate and that of soymilk do not have the same conditions, that are dissolving in water at low (less than about 0.5%) and high (more than about 3%) concentration. To make a more exact and wide calibration curve, soymilk that was changed in solid content was used. The protein content of soymilk was decided with the modified method of Bradford (Tezuka *et al.*, 2000). The absorbance of these bands had a high correlation with protein content. Particularly, the correlation between the absorbance of amide II band and protein content was higher than amide I band, because the latter overlaps the strong absorption of water near 1600 cm^{-1}. Even if the Δabs correction of water is done, the absorbance values of

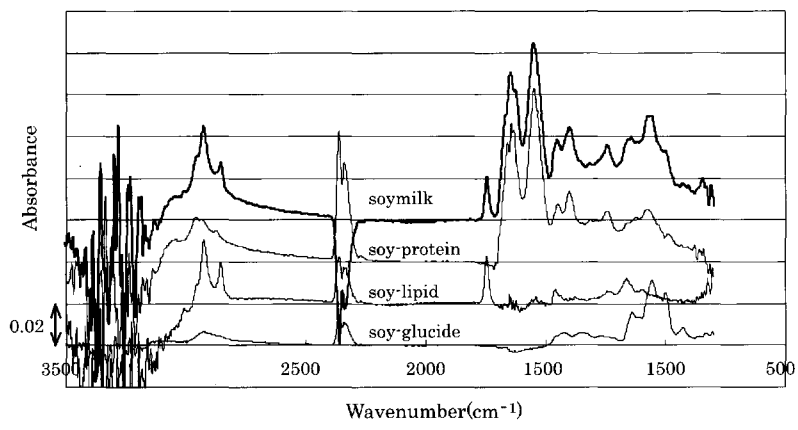

Fig. 3. IR spectra of soymilk and its components. IR spectra were measured from 4000 to 800 cm^{-1} with a FT-IR spectrophotometer equipped with an ATR apparatus. IR spectra of soymilk, soy-protein, soy-lipid, and soy-sugar are shown in order, from upper site. Soymilk was prepared from whole soybeans. Soy-protein was a solution prepared by the method of Thanh *et al.* (1975). Soy-lipid was prepared as a reconstituted oil-body formed by adding soybean oil to defatted soymilk and then treating it ultrasonically. Soy-sugar was the mixed solution of sucrose and stachyose.

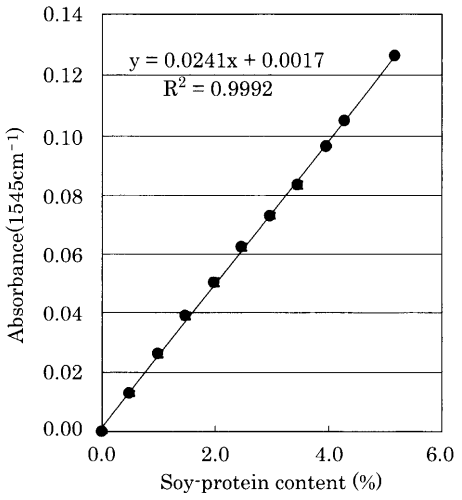

Fig. 4. The calibration curve of protein in soymilk. Absorbance of amide II band (1545 cm^{-1}) against soy-protein content is calibrated. Soymilk with changed solid content was used. The protein content of soymilk was determined by the modified method of Bradford. All points have error bars (standard deviation) of data (n=3), but the bar was hardly visible because of being so small.

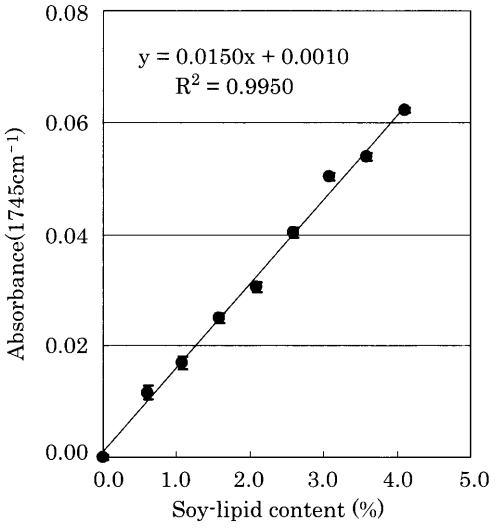

Fig. 5. The calibration curve of lipid in soymilk. Absorbance of ester band (1745 cm^{-1}) against soy-lipid content is calibrated. Soy-lipid was prepared as reconstituted oil-body formed by adding soybean oil to defatted soymilk and then treating it ultrasonically. All points have error bars (standard deviation) of data (n=3), but they are hardly visible because of being so small.

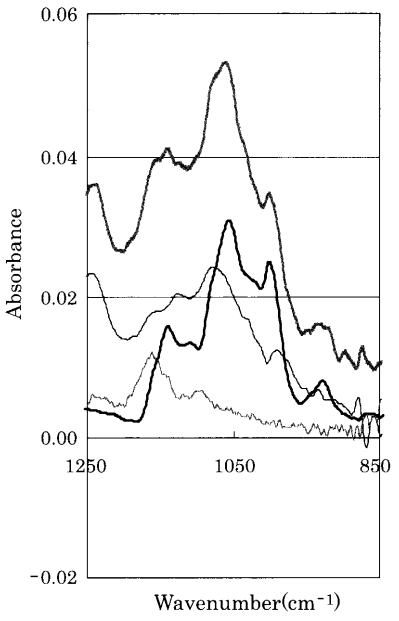

Fig. 6. IR spectra of soymilk and its components in C–C and C–O stretching band regions between 1200 and 900 cm^{-1}. Bold gray line, soymilk; bold black line, soy-sugar; simple black line, soy-protein; simple gray line, soy-lipid. The absorbencies of C–C and C–O bands from protein were smaller at 1000 cm^{-1} and from lipid were negligible.

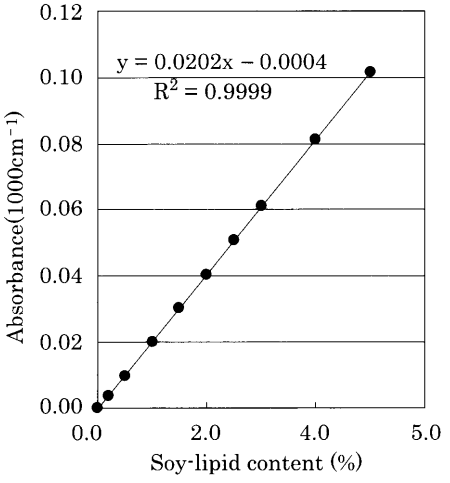

Fig. 7. The calibration curve of sugar in soymilk. Absorbance of C–C and C–O bands at 1000 cm^{-1} was calibrated against soy-sugar content. All points have error bars (standard deviation) of data (n=3), but they are hardly visible because they are so small.

amide I showed a low correlation to protein content (R^2>0.97). The absorbance of amide II band had a high correlation to protein content (R^2>0.99) as shown in Fig. 4. All points in Fig. 4 had error bars (standard deviation) of data, although the bar could hardly be seen because it was so small. The calibration curve for protein content in soymilk was obtained at 1545 cm^{-1} of amide II peak. The protein content in soymilk can be calculated directly from the absorption at 1545 cm^{-1}.

CH_2 and CH stretching bands (peaks at 2925 cm^{-1} and 2855 cm^{-1}) and ester band (peak at 1745 cm^{-1}) were remarkable in the spectrum of soy-lipid (reconstituted oil-body) solution as shown in Fig. 3. This spectrum was made by subtracting the IR-spectrum of defatted soymilk from that of reconstituted oil-body soymilk. Moreover, this spectrum was similar to that of soybean oil. The CH_2 and CH stretching band is attributed to lipid, protein, and sugar, whereas the ester band is ascribed to lipid, but not to protein and sugar. Therefore, the calibration curve for lipid content in soymilk was obtained at 1745 cm^{-1} of the ester band peak. The absorbance of the ester band had a high correlation with lipid content (R^2>0.99) as shown in Fig. 5. The lipid content in soymilk can be calculated directly from the absorption at 1745 cm^{-1}. All points in Fig. 5 had error bars (standard deviation) of data, but as in Fig. 4, it was hard to

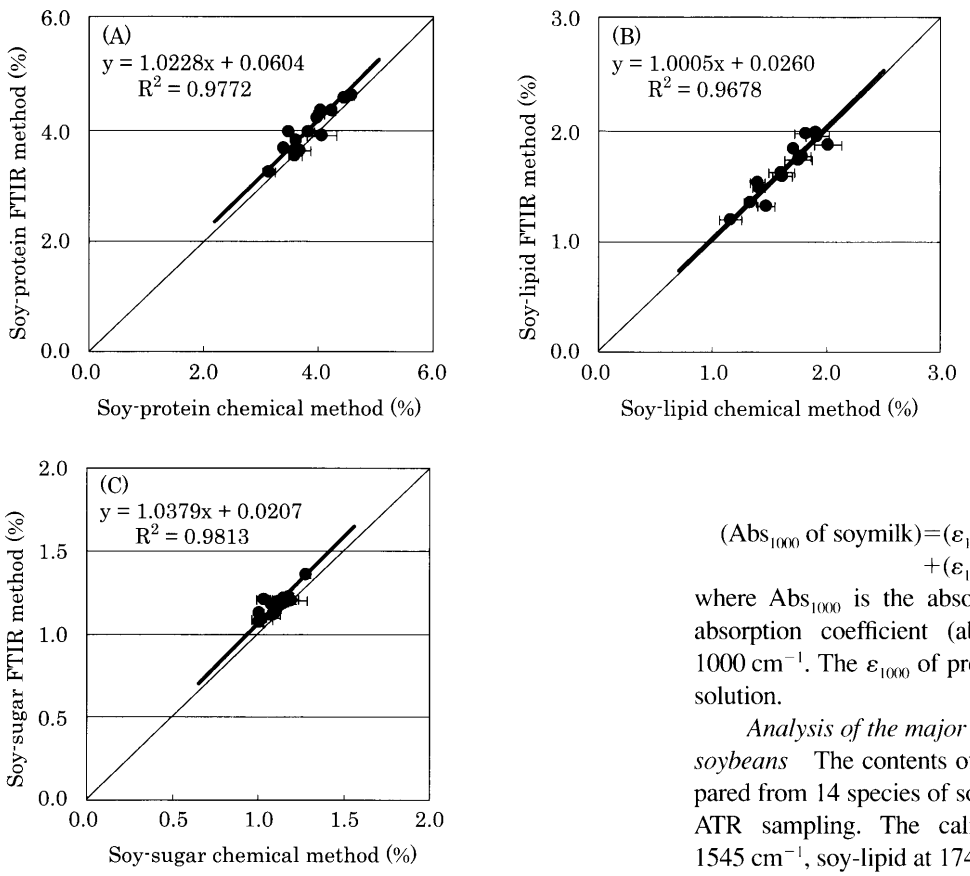

Fig. 8. Comparison of major component contents in soymilk by IR method with those by chemical method. Chemical methods of protein, lipid, and sugar are shown in Materials and Methods. IR methods can be detected in 0.01% viable order. The regression lines shown have slopes that are nearly one, intercepts that are nearly zero, and correlation coefficients that are nearly one. Even the difference of measurement was under 0.01% on FT-IR, although standard deviation was under 0.26% by the Bradford method (A), under 0.12% by the Soxhlet method (B), and under 0.13% by the ultrafiltration method (C).

identify because it was so small. Accurate measurement is needed to clarify the dispersed situation of lipid in water solution. The soymilks prepared from soybeans of 14 species had similar particle size of lipid dispersions. The main peak of the dispersions was 380 nm in diameter. Reconstituted soymilk for calibration of soy-lipid also had the same particle size of dispersion as those of soymilks. However, lipid-dispersion of raw soymilk (before heating) was different from that of soymilk. The raw soymilk showed different behaviors in the ester absorption band from soymilk (figure not shown). Therefore, this measurement of lipid content could not be adapted to that in raw soymilk.

C–C and C–O stretching bands (the region between 1200 and 900 cm^{-1}) appeared in the spectrum of soy-sugar solution (mixed solution of equal weights of sucrose and stachyose) as shown in Fig. 3. However, these bands are not only attributed to sugar, but also to protein and lipid as shown in Fig. 6. As the absorbance at the C–C and C–O bands from protein was smaller and that from lipid was negligible at low wavenumber, the peak of 1000 cm^{-1} was selected for sugar determination in C–C and C–O stretching bands (the region between 1200 and 900 cm^{-1}). Furthermore, absorbance of these bands had an extremely high correlation with sugar content (R^2>0.99) as shown in Fig. 7. All points in Fig. 7 had error bars (standard deviation) of data, but as in Figs. 4 and 5, they were hard to identify because they were so small. The sugar content in soymilk was then calculated by subtracting the effect of protein from the following equation (Eq. (1)).

$$(Abs_{1000} \text{ of soymilk}) = (\varepsilon_{1000} \text{ of protein}) \times (\text{protein}\%) + (\varepsilon_{1000} \text{ of sugar}) \times (\text{sugar}\%) \quad (1)$$

where Abs_{1000} is the absorbance at 1000 cm^{-1}, ε_{1000} is the absorption coefficient (absorbance per content%(w/w)) at 1000 cm^{-1}. The ε_{1000} of protein was obtained from soy-protein solution.

Analysis of the major components in soymilk from various soybeans The contents of major components in soymilk prepared from 14 species of soybeans were measured by FT-IR on ATR sampling. The calibration curves of soy-protein at 1545 cm^{-1}, soy-lipid at 1745 cm^{-1}, and soy-sugar at 1000 cm^{-1} (Figs.4, 5, and 7) were used for the determination. These chemical analysis results were also used for the purpose of comparison with FT-IR results and are shown in Fig. 8. In these results, the values by FT-IR method were nearly equal to the values by chemical analysis. It seems that these chemical analysis data had larger error than FT-IR data, because the separation and isolation process for determination was accompanied by a mass loss and a few impurities. Even the difference of measurement is under 0.01% on FT-IR, although standard deviation is under 0.26% on the Bradford method, under 0.12% on the Soxhlet method, and under 0.13% on the ultrafiltration method. This mid-IR method can be done without any separation and isolation process, and therefore is useful for limited measurement (percentage concentration between 1 and 6) of the major components (protein, lipid, and sugar) in soymilk.

Conclusion

Fourier-transform infrared spectroscopy (FT-IR) at mid-infrared on attenuated total reflectance (ATR) sampling has been used for the quantitative analysis of the major components (protein, lipid, and sugar) in soymilk without any sample separation or complicated statistical computation. This method is simpler and more rapid than conventional ones.

Acknowledgment Part of this work was supported by financial aid from the research project "Selective breeding for high-quality products and development of new products" from the Ministry of Agriculture, Forestry and Fisheries of Japan.

References

Albert, T.P., Nabetani, H., Sessa, D.J., Wolf, W.J., Liebman, M.N. and Dukor, R.K. (1996). Effects of bound water on FTIR spectra of glycinin. *J. Agric. Food Chem.*, **44**, 2220–2224.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

Briandet, R., Kemsley, E.K. and Wilson, R.H. (1996). Discrimination of arabica and robusta in instant coffee by Fourier transform infrared spectroscopy and chemometrics. *J. Agric. Food Chem.*, **44**, 170–174.

Defernez, M., Kemsley, E.K. and Wilson, R.H. (1995). Use of infrared spectroscopy and chemometrics for the authentication of fruit purees. *J. Agric. Food Chem.*, **43**, 109–113.

Edelmann, A., Diewok, J., Schuster, K.C. and Lendl, B. (2001). Rapid method for the discrimination of red wine cultivars based on mid-infrared spectroscopy of phenolic wine extracts. *J. Agric. Food Chem.*, **49**, 1139–1145.

Guillen, M.D. and Cabo, N. (1999). Usefulness of the frequency data of the Fourier transform infrared spectra to evaluate the degree of oxidation of edible oils. *J. Agric. Food Chem.*, **47**, 709–719.

Iizuka, K. and Aishima, T. (1999). Differentiation of sauce by pattern recognition analysis of mid- and near-IR spectra. *J. Food Comp. Anal.*, **12** 197–209.

Iizuka, K. and Aishima, T. (1999). Starch gelation process observed by FT-IR/ATR spectrometry with multivariate data analysis. *J. Food Sci.*, **64**, 653–658.

Jowder, O.A., Defernez, M., Kemsley, E.K. and Wilson, R.H. (1999). Mid-infrared spectroscopy and chemometrics for the authentication of meat product. *J. Agric. Food Chem.*, **47**, 3210–3218.

Kemsley, E.K., Holland, J.K., Defernez, M. and Wilson, R.H. (1996). Detection of adulteration of raspberry purees using infrared spectroscopy and chemometrics. *J. Agric. Food Chem.*, **44**, 3864–3870.

Kemsley, E.K., Tapp, H.S., Scarlett, A.J., Miles, S.J., Hammond, R. and Wilson, R.H. (2001). Comparison of spectroscopic techniques for the determination of Kjeldahl and ammoniacal nitrogen content of farmyard manure. *J. Agric. Food Chem.*, **49**, 603–609.

Lai, Y.W., Kemsley, E.K. and Wilson, R.H. (1994). Potential of Fourier transform infrared spectroscopy for the authentication of vegetable oils. *J. Agric. Food Chem.*, **42**, 1154–1159.

McQueen, D.H., Willson, R.H. and Kinnunen, A. (1995). Near and mid-infrared photoacoustic analysis of principal components of foodstuffs. *Trends Anal. Chem.*, **14**, 482–492 .

Ono, T., Takeda, M. and Guo S.T. (1996). Interaction of protein particles with lipids in soybean milk. *Biosci. Biotechnol. Biochem.*, **60**, 1165–1169.

Tezuka, M., Taira, H., Yagasaki, K. and Ono, T. (2000). Properties of soy milks prepared from soybeans having different subunits of glycinin. *J. Agric. Food Chem.*, **48**, 1111–1117.

Thanh, V.H., Okubo, K. and Shibasaki, K. (1975). Isolation and characterization of the multiple 7S globulins of soybean proteins. *Plant Physiol.*, **56**, 19–22.

Tzen, J.T.C. and Huang, A.H.C. (1992). Surface structure and properties of plant seed oil bodies. *J. Cell Biol.*, **117**, 327–335.

Willson, R.H. and Tapp, H.S. (1999). Mid-infrared spectroscopy for food analysis: recent new applications and relevant developments in sample presentation methods. *Trends Anal. Chem.*, **18**, 85–93.