The Effect of Freeze-Drying Pretreatment on the Accuracy of Near Infrared Spectroscopic Food Analysis to Predict the Nutritive Values of Japanese Cooked Foods

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Summary The official testing methods for establishing nutritive values are accurate but relatively costly and time-consuming. Near infrared spectroscopy (NIRS) is potentially an alternative method that can analyze several components in a few minutes using an exclusively electronic instrument with no need for a laboratory expert. However, the accuracy of commercial NIRS spectroscopic food analyzers is not sufficient for Japanese food labeling, because of interference from moisture contained in the foods. This study aims to assess the effect of a freeze-drying pretreatment on the accuracy of NIRS food analysis. Thirty-four samples, consisting of six food items habitually consumed in Japan and cooked by different cooking methods were treated by milling then freeze-drying. They were analyzed by a commercial NIRS instrument (Calorie Answer™) with calibration curves developed based on other freeze-dried samples. The obtained nutritive values (energy, protein, lipid, carbohydrate and moisture) were corrected to the values before freeze-drying using the vaporized moisture content. The same samples before freeze-drying were also analyzed using the official testing methods to assess the analytical accuracy using NIRS after freeze-drying, and further analyzed using the same NIRS with the commercial calibration curves to assess the effect of freeze-drying. The accuracies were better for the freeze-dried samples than for the wet samples. The magnitude of the error in energy and carbohydrate was significantly associated with the retained moisture content in the freeze-dried sample. In conclusion, freeze-drying was an effective pretreatment for improving the accuracy of NIRS analyses of Japanese cooked foods, although it is still time-consuming and needs additional investment.

Key Words food labeling, Calorie Answer™, energy, moisture, simplified method

Food labeling is an effective tool to help protect consumer health (1). The Japanese Food Labeling Act requires all processed foods and additives to display nutritional facts regarding energy, protein, lipid, carbohydrate, and salt equivalent as a minimum on the labels of foods offered for sale (2). It is recommended that these nutritive values should be obtained using the official testing methods. However, these methods are relatively costly, time-consuming, and less sustainable environmentally for the manufacturers who are responsible for complying with these labeling requirements.

Near infrared spectroscopy (NIRS) is one method capable of simultaneously analyzing several components of samples without weighing, treatment with chemicals, or discharging large amounts of waste. When a sample is irradiated with near infrared (NIR) light of wavelengths between about 730 and 2,500 nm, the light is absorbed selectively according to the specific vibrational frequencies of the molecules present and gives rise to a particular spectrum (3, 4). Spectral data arising from overlapping wavelengths are unique to a sample, as they include information related to the chemical and physical properties of the organic molecules in the sample. To remove background data and noise as well as to distinguish different components with overlapping absorption peaks, the data are mathematically preprocessed then statistically processed, allowing the components to be identified and quantified using prepared calibration curves (3, 4). The samples can be prepared and analyzed by an operator with only brief training so a highly-trained laboratory expert is not needed. The analysis takes only a few minutes with running costs incurred only for electricity. The samples are unaffected by this analysis so can be reused instead of being discarded. Overall, NIRS is superior in terms of...
the time required for analysis, running costs, operational flexibility and environmental sustainability, thus enabling frequent and repeated analyses. This method has already been applied practically in the food industry (5–7).

However, it has been reported that NIRS is not suitable for accurately quantifying nutrients in unhomogenized foods (4) and in moisture-rich foods (4) at levels lower than the NIR spectrometers can accurately detect (7) or for protein (4), especially in lipid-rich foods (8). These disadvantages have been already observed when analyzing ordinary Japanese cooked foods using commercial NIRS food analyzers (9).

Potential solutions to these problems include freeze-drying the samples to eliminate the interference of moisture (10). It also helps to homogenize the sample finely, reducing the errors arising from variations in temperature (10), and concentrating nutrients to detectable levels. The aim of the present study is to assess the effect of freeze-drying on the accuracy of determining the nutritive values of Japanese cooked foods using commercial NIRS food analyzers (9).

Table 1. Food items and cooking methods of the sample.

| Cooking method | Food items (n = 34) |
|----------------|---------------------|
|                | Potato (n = 4)      |
| Raw (n = 6)    | - Raw              |
| Steam (n = 6)  | - Steamed          |
| Boil/stew (n = 5) | - Stewed with miso |
| Roast/grill/bake (n = 3) | - Roasted |
| Stir-fry/Sauté (n = 5) | - Sautéed |
| Fry (n = 9)    | - French-fried     |

Materials and Methods

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Materials and Methods

Samples. Thirty-four samples were prepared, consisting of six food items habitually consumed in Japan using six common cooking methods (Table 1). All food items were purchased between August and December 2015. Each sample was milled into particles with a diameter of 2–3 mm using a Cuisinart FP-25 kitchen blender (Cuisinart San-ei, Co. Ltd., Tokyo, Japan), then divided into three batches: for the freeze-dried samples (D), the wet samples (W) and the samples to obtain the reference values (Ref). The samples in the D group were freeze-dried at 240˚C or less and at 0.133 mb or less using a FreeZone 12 Liter Freeze Dry System (Model 77540, Labconco, Kansas City, MO, USA) connected to a FreeZone Stoppering Tray (Model 79480, Labconco) for approximately 2 wk until their weight had become constant, then ground to particles of less than 1 mm in diameter using a mortar and pestle. The change in weight during freeze-drying was recorded as the vaporized moisture content. The samples in the Ref group were analyzed to obtain the reference values, using the official testing method regulated by the Japanese Nutrition Labeling Standard (11), at the Japan Frozen Food Inspection Corporation (Tokyo, Japan). The retained moisture content was calculated as the difference between the reference value and the vaporized content of moisture.

NIR analysis. The NIR spectroscopic food analyzer Calorie Answer™ (Model CA-HM, Joy World Pacific Co. Ltd., Aomori, Japan) was used for the analyses of the samples in the D and W groups. This analyzer irradiated the samples using halogen lamps as the radiation source, to obtain NIR absorbance spectra over a wavelength ranging from 1,100 to 2,200 nm at intervals of 2 nm from the surface to a depth of several mm (12).
The analyzer was connected to a personal computer running JWP CA-HM Client software (Joy World Pacific Co. Ltd.) to record the NIR absorbance spectral data obtained from the samples. Readings were taken at least 30 min after switching on the analyzer to ensure stable conditions. The samples in the D and W groups were allowed to come to room temperature (23 ± 2°C) for at least 30 min as advised in the analyzer operating manual. The cylindrical sample cell for reflectance mode (internal diameter = 50 mm, depth = 10 mm) was filled with the sample then covered with a quartz glass coverslip to flatten the sample surface before placing it in the sample holder. Each analysis was performed following instrument adjustment using a standard cell. The analyses took about 5 min and were made in triplicate.

**Developing calibration equations for dried samples.** The samples used in a previous study of Japanese foods (9) had been freeze-dried and stored in the same manner as the present study. Ninety samples contributed their nutritive values by technical experts with no knowledge of the reference values. The fatty samples in the D group were treated using the PL mode: mackerel (all items), pork (all items), chicken (all items), potato (French-fried and breaded & fried), eggplant (sautéed, braised, battered & fried, and breaded & fried), and pancake mix (doughnut), whereas the other samples in the D group were treated using the Normal mode. The samples in the W group were treated using the optimal commercial calibration procedure available in the CA-HM Client software depending on the sample properties corresponding to one of the twelve measurement settings: for grains, fresh light-colored vegetables, cooked potatoes, raw fish, cooked fish, raw or cooked meats, seasoned meats, sweet buns, salads, stewed foods, fried foods, and processed foods.

The calibration data sets were collected from triplicate analytical data of the samples used for the study of Japanese cooked foods (9) stored in the freeze-dried condition. SE: standard error of the mean. RMSEC: root mean square of error of calibration. RPDc: ratio of prediction to deviation in calibration. R²c: coefficient of determination of calibration. PL: the calibration curve for lipid-rich foods being developed using the absorbances at wavelengths closely related to the protein and lipid profile.

### Table 2. Statistical overview of the calibration data sets.

| Mode   | Nutritive value | Sample | n   | mean±SE (per 100 g) | min | max | RMSEC | RPDc | R²c |
|--------|-----------------|--------|-----|---------------------|-----|-----|-------|------|-----|
| PL     | Energy          | 47     | 136 | 501±5 (kcal)        | 364 | 660 | 20    | 3.2  | 0.95 |
|        | Protein         | 47     | 136 | 27.2±1.4 (g)       | 5.2 | 89.2| 4.5   | 3.9  | 0.97 |
|        | Lipid           | 47     | 136 | 27.0±1.0 (g)       | 4.3 | 58.2| 3.0   | 4.0  | 0.97 |
|        | Carbohydrate    | 39     | 101 | 36.7±1.8 (g)       | 0.7 | 71.9| 2.2   | 8.7  | 0.99 |
|        | Moisture        | 39     | 111 | 1.9±0.1 (g)        | 0.6 | 4.9 | 0.4   | 2.3  | 0.90 |
| Normal | Energy          | 35     | 101 | 402±3 (g)          | 332 | 471 | 11    | 3.3  | 0.95 |
|        | Protein         | 32     | 95  | 13.2±0.4 (g)       | 5.3 | 22.4| 1.2   | 3.5  | 0.96 |
|        | Lipid           | 35     | 101 | 6.7±0.4 (g)        | 1.5 | 17.5| 1.0   | 4.5  | 0.98 |
|        | Carbohydrate    | 35     | 101 | 71.2±0.8 (g)       | 50.2| 90.3| 2.3   | 3.7  | 0.96 |
|        | Moisture        | 32     | 84  | 1.8±0.1 (g)        | 0.5 | 5.4 | 0.3   | 3.3  | 0.95 |

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The recorded absorbances of the samples in the D and W groups were transformed into nutritive values by technical experts with no knowledge of the reference values. The fatty samples in the D group were treated using the PL mode: mackerel (all items), pork (all items), chicken (all items), potato (French-fried and breaded & fried), eggplant (sautéed, braised, battered & fried, and breaded & fried), and pancake mix (doughnut), whereas the other samples in the D group were treated using the Normal mode. The samples in the W group were treated using the optimal commercial calibration procedure available in the CA-HM Client software depending on the sample properties corresponding to one of the twelve measurement settings: for grains, fresh light-colored vegetables, cooked potatoes, raw fish, cooked fish, raw or cooked meats, seasoned meats, sweet buns, salads, stewed foods, fried foods, and processed foods.

#### Data processing.

The recorded absorbances of the samples in the D and W groups were transformed into nutritive values by technical experts with no knowledge of the reference values. The fatty samples in the D group were treated using the PL mode: mackerel (all items), pork (all items), chicken (all items), potato (French-fried and breaded & fried), eggplant (sautéed, braised, battered & fried, and breaded & fried), and pancake mix (doughnut), whereas the other samples in the D group were treated using the Normal mode. The samples in the W group were treated using the optimal commercial calibration procedure available in the CA-HM Client software depending on the sample properties corresponding to one of the twelve measurement settings: for grains, fresh light-colored vegetables, cooked potatoes, raw fish, cooked fish, raw or cooked meats, seasoned meats, sweet buns, salads, stewed foods, fried foods, and processed foods.

#### Data correction for the freeze-dried samples.

Each analytical nutritive value per 100 g (dry weight) obtained from the samples in the D group was corrected to the value per 100 g (wet weight) using the vaporized moisture content during freeze-drying (13) and the follow-
Equation (1) was for energy, protein, lipid and carbohydrate, and equation (2) was for moisture:

\[ N_{\text{w}} = N_d / 100 \times (100 - V_w) \]
\[ M_{\text{w}} = U_d / 100 \times (100 - V_w) + V_w \]

where \( N_{\text{w}} \) is the nutritive value per 100 g (wet weight), \( N_d \) the nutritive value per 100 g (dry weight), \( V_w \) the vaporized moisture content per 100 g (wet weight), \( M_w \) the total moisture content per 100 g (wet weight) and \( U_d \) the moisture content per 100 g (dry weight). As freeze-drying cannot completely remove moisture from normal foods (14), the moisture content per 100 g (wet weight) was calculated as the total of the vaporized moisture content \( (V_w) \) per 100 g (wet weight) and the analytical moisture content \( (U_d) \) converted to per 100 g (wet weight).

**Evaluation.** The D group was compared with the Ref group to assess the accuracy of NIR analysis after the freeze-drying pretreatment, as well as with the W group to assess the effect of freeze-drying on the accuracy of the NIR analysis. The ratio of prediction to deviation (RPD) and the proportion conforming to the Japanese Nutrition Labeling Standard \( (\% n_t) \) were used as indices of accuracy. RPD is a non-dimensional statistic for the rapid evaluation of predictive ability of an NIRS calibration model (15). It was calculated as follows:

\[ \text{RMSEP} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n}} \]
\[ \text{RPD} = \text{sd/RMSEP} \]

where RMSEP is the root mean square of error of prediction, \( \hat{y}_i \) the analytical value, \( y_i \) the reference value, \( n \) the number of analytical values, and \( \text{sd} \) the standard deviation of the reference values. For complicated materials such as forage, feeds, soils, and functional factors, the values of RPD less than 2.0, from 2.0 to 2.4, 2.5 to 2.9, 3.0 to 3.4, 3.5 to 4.0, and equal or greater than 4.1 were respectively defined as “Very poor”: not recommended for any application, “Poor”: applicable for rough screening, “Fair”: applicable for screening, “Good”: applicable for quality control, “Very Good”: applicable for process control, and “Excellent”: applicable for any application (15). The values of \( \% n_t \) were calculated as follows:

\[ \% n_t = n/n_i \]

where \( n_i \) is the number of analytical values within the tolerance allowances required by the Japanese Nutrition Labeling Standard as \( \{ \text{value measured using the official testing method}/(\text{labeled value}) - 1 \} \) within \( \pm 20\% \), and \( n \) is the number of analytical values. This standard allows a tolerance range of \( \pm 5 \) kcal for foods with energy contents less than 25 kcal per 100 g or 100 mL, and a tolerance range of \( \pm 0.5 \) g for foods with macronutrient contents less than 2.5 g per 100 g or 100 mL to avoid excessive regulation. The value of \( \% n_t \) has not been calculated for moisture because no tolerance allowance has been specified.

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**Table 3. Evaluation of nutritive values.**

| Pretreatment | \( n \) | Nutritive value | Difference from reference value | RMSEP | RPD | \( R^2 \) | \( \% n_t \) |
|-------------|-----|----------------|---------------------------------|------|-----|------|--------|
|             |     | Median (25th, 75th percentiles) | Median (%) | 95% CI |      |      |        |
| Energy (kcal/100 g) |     |                          |                  |       |      |      |        |
| Ref         | 34  | 218 (166, 320)          | 7 (3.0%)         | 0–10  | 14  | 7.4  | 0.98  | 97%   |
| D           | 34  | 226 (161, 326)          |                  |       |      |      |        |
| W           | 34  | 216 (179, 258)          | 4 (1.6%)         | −33–4 | 54  | 1.9  | 0.76  | 68%   |
| Protein (g/100 g) |     |                          |                  |       |      |      |        |
| Ref         | 34  | 19.6 (3.1, 22.5)        | 0.3 (1.5%)       | −0.3–1.3 | 2.3 | 4.1  | 0.95  | 79%   |
| D           | 34  | 20.3 (2.5, 23.5)        |                  |       |      |      |        |
| W           | 34  | 14.5 (5.7, 18.5)        | −2.3 (−11.5%)    | −3.7–0.4 | 5.2 | 1.9  | 0.77  | 44%   |
| Lipid (g/100 g) |     |                          |                  |       |      |      |        |
| Ref         | 34  | 12.4 (5.3, 20.9)        | −0.1 (−0.8%)     | −1.2–0.2 | 1.9 | 5.2  | 0.97  | 82%   |
| D           | 34  | 11.3 (6.5, 21.9)        |                  |       |      |      |        |
| W           | 34  | 13.6 (6.5, 18.6)        | −0.2 (−1.2%)     | −3.4–0.1 | 5.1 | 2.0  | 0.78  | 41%   |
| Carbohydrate (g/100 g) |     |                          |                  |       |      |      |        |
| Ref         | 34  | 4.8 (0.0, 11.1)         | 1.3 (26.0%)      | 0.7–2.9 | 3.5 | 3.5  | 0.95  | 68%   |
| D           | 34  | 7.2 (1.7, 13.5)         |                  |       |      |      |        |
| W           | 34  | 5.9 (0.3, 16.8)         | 0.4 (7.3%)       | 1.0–3.3 | 3.9 | 3.2  | 0.96  | 74%   |
| Moisture (g/100 g) |     |                          |                  |       |      |      |        |
| Ref         | 34  | 57.8 (49.0, 71.8)       | −1.4 (−2.3%)     | −2.0–0.7 | 2.4 | 6.7  | 0.99  |       |
| D           | 34  | 57.4 (46.1, 71.0)       |                  |       |      |      |        |
| W           | 34  | 63.9 (56.8, 67.4)       | 0.4 (0.7%)       | −1.1–4.7 | 8.5 | 1.9  | 0.72  |       |

RMSEP: root mean square of error of prediction. RPD: ratio of prediction to deviation. \( R^2 \): coefficient of determination of prediction. Ref: reference value by official testing method. D: freeze-dried sample analyzed by NIR spectrometer. W: wet sample analyzed by NIR spectrometer. \( \% n_t \), the proportion conforming to the Japanese Nutrition Labeling Standard.
Statistics. The data are presented as median and 25th and 75th percentiles. The Shapiro-Wilk test was used to confirm the normality of the distributions. The effects of the food items and cooking methods on accuracy were examined using within/between analysis of variance (ANOVA) with (analysis method) x (food item or cooking method) as an interaction, followed by Bonferroni’s correction when a significant interaction was observed. The association between the retained moisture content and the difference from the reference value was tested by Spearman’s rank correlation coefficient. The statistical analysis was performed using SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA). The α level for significance was set at p < 0.05.

RESULTS

The analytical nutritive values and the indices of accuracy are shown in Table 3. The association between the analytical value and the reference value, and between the retained moisture content and the difference from the reference value was tested by Spearman’s rank correlation coefficient. The statistical analysis was performed using SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA). The α level for significance was set at p < 0.05.

A significant interaction between analysis methods and food items was observed for energy and moisture contents (Table 4). After applying Bonferroni’s post hoc correction, significant differences between the D group and Ref group were observed for potato (energy and moisture), mackerel (energy and moisture), chicken (energy), pork (moisture) and pancake mix (energy and moisture). A significant interaction between analysis methods and cooking methods was observed for moisture only (Table 4). After applying Bonferroni’s post hoc correction, significant differences between the D group and Ref group were observed for “Boil/stew” (moisture) and “Roast/grill/bake” (moisture).
Table 4. Comparison of nutritive values by the analysis method and food item or cooking method.

| Food            | Analysis | Energy (kcal) | Protein (g) | Lipid (g) | Carbohydrate (g) | Moisture (g) |
|-----------------|----------|---------------|-------------|-----------|------------------|--------------|
| Potato (n=4)    | Ref      | 128±36        | 2.4±0.5     | 3.5±2.1   | 21.7±3.7         | 71.4±6.4     |
| Chicken (n=7)   | D        | 140±37*       | 1.7±0.2     | 4.3±2.4   | 23.7±3.8         | 69.8±6.5*    |
| Pork (n=8)      | Ref      | 211±18        | 22.6±1.0    | 12.3±1.9  | 2.5±1.6          | 60.3±2.7     |
| Pancake mix (n=3) | D     | 226±17*       | 23.6±1.1    | 13.1±1.5  | 3.2±1.5          | 58.4±2.6*    |
| Eggplant (n=6)  | Ref      | 87±26         | 1.8±0.3     | 5.5±2.2   | 7.6±1.3          | 84.5±3.7     |
| Mackerel (n=6)  | D        | 87±24         | 2.1±0.4     | 5.1±2.0   | 8.2±1.3          | 83.7±3.7     |
| Cooking method  | Analysis |    |             |           |                  |              |
| Raw (n=4)       | Ref      | 234±19        | 22.1±1.3    | 14.3±1.7  | 4.0±1.6          | 57.7±2.8     |
| Steam (n=6)     | D        | 224±19*       | 22.6±1.1    | 13.1±1.7  | 4.0±1.6          | 58.7±2.9     |
| Boil/stew (n=5)| D        | 343±19        | 20.9±0.9    | 28.1±2.1  | 1.6±1.0          | 48.1±2.5     |
| Roast/grill/bake (n=3)| D | 349±21        | 23.3±1.5    | 26.4±2.3  | 5.6±1.8          | 45.5±2.5*    |
| Fry (n=9)       | Ref      | 283±41        | 6.7±0.3     | 10.1±5.0  | 41.3±1.9         | 40.7±4.3     |

Data are presented as mean±SE. Within/between analysis of variance (ANOVA) with (analysis method)×(food item or cooking method) as an interaction was used to examine the accuracy. * indicates a significant difference from Ref group in each food item or cooking method by Bonferroni’s correction when a significant interaction was observed for each nutritive value (p<0.05). Ref: reference value by official testing method. D: freeze-dried sample analyzed by NIR spectrometer.

DISCUSSION

The accuracy of prediction has been improved remarkably through using the freeze-drying pretreatment based on the indices of accuracy. A number of factors were responsible for this: freeze-drying removing moisture from the samples and hence interference with the NIR absorbance spectra; weighing the vaporized moisture directly that occupies most of the moisture contained in a sample; improving the homogenization of samples by grinding into a powder form; and eliminating the biased concentration of fats and oils on the irradiated surface, which often occurs on a wet sample. Using two calibration curves, based on the freeze-dried samples to overcome the interference of lipid in quantifying protein, also helped to improve accuracy.

A disadvantage of freeze-drying is that it needs expensive equipment and requires a significant processing time. Therefore, the application of other drying methods is also worth considering. The official testing methods for moisture, which have also been used for the food component tables in Japan (16), may be con-
Energy and macronutrients were also affected by the reference values, so that the RPD values would also be more widely distributed. This aspect should be examined using other samples where the carbohydrate contents have been smaller. This suggests that problems with accuracy would still remain with foods containing very little or none of a targeted nutrient. Moreover, the relatively low RPD for carbohydrate may have also been affected by the reference values distribution. RPD, the ratio of the standard deviation of the reference values to RMSEP, is likely to have been lower because of the narrow range of variation in the reference values (23). The reference values for carbohydrate in the present study were biased towards the lower range as it included nine 0 g/100 g values in the meat and fish data, leading to a smaller standard deviation in the reference values, so that the RPD values would also have been smaller. This aspect should be examined using other samples where the carbohydrate contents are more widely distributed.

Limitations
The range of food items and cooking methods examined were not wide enough to represent the complete diversity of Japanese meals. Further studies on a wider range of samples of Japanese food will be needed to confirm our findings.

Accuracy of energy and macronutrients
The analyzer used in the present study uses a single energy conversion factor for carbohydrate regardless of whether it is energetic or non-energetic, which would lead to overestimating the energy content of foods independent of other factors. The magnitude of this error may be significant for low energy foods containing a relatively high content of dietary fiber such as the steamed eggplant examined in the present study.

A previous study analyzing human milk has reported that protein was quantified more accurately by removing fat from the sample using centrifugation (8). This suggests that high concentrations of lipids may be responsible for errors in quantifying protein using NIRS. In the present study, this problem has been addressed to some extent by using two types of calibration curve: one for foods rich in lipid and one for the other foods. However, the basis for deciding which of the two calibration curves should be used for the target food before analysis still needs to be determined.

Lipid is a food component that can be quantified with a relatively high accuracy using NIRS (3), regardless of whether it had been dried or not. A previous study reported that the accuracy of determining the lipid content of freeze-dried cheese was lower than that of fresh cheese analyzed by the same NIR spectrometer (22). The accuracy was higher for the freeze-dried samples than the wet samples in the present study, one of the possible reasons being that the calibration curves and the data correction method may be better suited to the freeze-dried samples.

The D group was slightly inferior to the W group in terms of the coefficient of determination and %RSD for carbohydrate. This might have been caused by overfitting of the calibration curves exhibiting high RPD for carbohydrate during calibration and also due to the limit of quantification. The analytical values for carbohydrate obtained from the freeze-dried samples containing less than 10 g/100 g of carbohydrate tended to be outside the tolerance allowance. This suggests that problems with accuracy would still remain with foods containing very little or none of a targeted nutrient. Moreover, the relatively low RPD for carbohydrate may have also been affected by the reference values distribution. RPD, the ratio of the standard deviation of the reference values to RMSEP, is likely to have been lower because of the narrow range of variation in the reference values (23). The reference values for carbohydrate in the present study were biased towards the lower range as it included nine 0.0 g/100 g values in the meat and fish data, leading to a smaller standard deviation in the reference values, so that the RPD values would also have been smaller. This aspect should be examined using other samples where the carbohydrate contents are more widely distributed.

The impact of retained moisture on accuracy
There were significant positive associations between the magnitude of the errors and the retained moisture content for energy and carbohydrate. This suggested that at least some of the retained moisture may not have been quantified as moisture but as carbohydrate thus the energy derived from carbohydrate would have been overestimated. This retained moisture in the freeze-dried samples could be identified as “bound water” (14) which is bound tightly to constituents and cannot be frozen or vaporized. As the NIR absorbance spectrum of bound water is different from that of free water (19), the analyzer might be unable to quantify it accurately. Such errors may be more pronounced when foods with a high bound water content were analyzed, for example, jams, dumplings, or foods containing high levels of soluble dietary fiber. A future task will be to quantify bound water as a part of moisture more accurately. Such errors may be more pronounced when foods with a high bound water content were analyzed, for example, jams, dumplings, or foods containing high levels of soluble dietary fiber. A future task will be to quantify bound water as a part of moisture more accurately.

The impact of food items and cooking methods
Significant interactions were observed between the analysis method and the food item for energy and moisture. This was possibly caused by the differences in retained moisture content described above, because significant differences between the analytical values and the reference values were observed for both energy and moisture in potato, mackerel, and pancake mix.

A significant interaction between the analysis method and the cooking method was observed only for moisture. This suggested that the cooking method may influence the accuracy of moisture analysis, but was not large enough to affect the accuracy of predicting the other nutrients which are displayed on food labels.

Accuracy of energy and macronutrients
The analyzer used in the present study uses a single
CONCLUSION

Freeze-drying was an effective pretreatment before analyzing samples of Japanese cooked foods. It helped to provide more accurate analyses using NIRS. Although this procedure requires additional equipment and a longer preparation time, it retains the advantages of low analysis costs, operational flexibility and environmental sustainability.

Authorship

Research conception and design: KIT and YH; experiments: KIT, CT, and FM; preparation of calibration equations: NK, YI, and SS; statistical analysis of the data: YH; writing of the manuscript: YH and KIT. All authors contributed to the interpretation of the results and read and approved the final manuscript.

Disclosure of state of COI

KIT has received the financial support from Joy World Pacific Co. Ltd. in addition to the preparation of calibration equations for dried samples. However, they did not participate with the data analysis.

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