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Original Article

Development of a New Simple Estimating Method for Protein, Fat, and Carbohydrate in Cooked Foods.

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Abstract

Evaluations of daily nutrient intakes with practical accuracy contribute not only to public but also to personal health. To obtain accurate estimations of nutrient intake, chemical analyses of a duplicate sample of all foods consumed are recommended. But these analytical methods are expensive, time consuming, and not practically applicable for field surveys dealing with numerous food samples. To solve this problem, a new rapid and simple method of estimating nutrients is developed here. Elemental compositions of cooked foods were examined using a high speed and high performance carbon, hydrogen, and nitrogen autoanalyzer, and the results showed good reproducibility. A significant correlation between Kjeldahl's and the autoanalyzer methods was observed in the nitrogen measurement (n=20; r =0.999; p< 0.001), and very good agreement was observed between the methods. Therefore, the nitrogen amount obtained by the autoanalyzer was used for the estimation of the protein proportion in the cooked foods. The fat and carbohydrate proportions estimated by the new method correlated with the values obtained by the chemical method (p< 0.001 each). There were also good agreements of fat and carbohydrate proportions between the chemical and the new estimation methods. According to these results, the new, rapid and simple estimation method established in this study should be applicable to nutritional research.

Key words: duplicate portion sampling technique, nutrients, nutritional survey

Introduction

Because nutrition is one of the most important factors in human growth, development, and health maintenance, accurate evaluations of normal daily intakes of nutrients are important [1]. The connection between diet and both general health status and specific diseases has been widely popularized in industrialized countries [2]. Specific types of foods or patterns of eating are thought to be healthy, or desirable, and others are considered unhealthy, or undesirable [3]. However, the difficulty of quantifying dietary intake has long been recognized as a problem by researchers and clinicians [4]. Dietary surveys can be used in a number of ways depending on the aim of the particular study [5], and the strengths and weaknesses of the various survey methods have been widely discussed [6]. However, considerable differences of nutrient intake have been observed among survey methods [7-10]. It is essential to minimize the causes of the variation between actual and measured diets [11].

Accurate evaluations of nutrient intakes are problematic [4]. Pekkarinen [12] and Marr [13] claim that there is no definitive method for such evaluations, but both agree that taking a duplicate sample of all foods consumed and analyzing these duplicates in a laboratory is likely to provide the most accurate information. However, chemical analysis of the nutrient content of foods is an expensive and time-consuming undertaking beyond the scope of most research [7, 14].

Despite the recent use of sophisticated statistical analyses and the increased variability of the modern diet, conclusions on the methodology of nutritional estimates have changed very little [10]. In this paper, a rapid, simple, and reliable method of estimating nutrients in cooked foods is presented using a carbon, hydrogen, and nitrogen autoanalyzer that has been used formerly for the analysis of organic compounds. After examining the reproducibility of measurements in food samples, a new estimation method was contrived to estimate the protein, fat, and carbohydrate proportions from elemental compositions of food samples. Nutrient proportions estimated by the new method were compared to those estimated by the traditional chemical methods [15, 16].

Materials and Methods

Food sampling.

All food samples were collected in cafeterias and restaurants in a medical university. The food samples were divided into two groups: 1) individual dishes (n = 60), i,e., cooked rice, meat, fish,

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or vegetables with seasonings; and 2) lunches (n = 60) consisting of rice or bread, meat, fish, vegetables, and fruits. The food samples were collected in plastic sealing boxes, which were weighed and cleaned. After removal of inedible material, e.g., bones and seeds, samples were homogenized using an electrical mixer with suitable amounts of distilled deionized water. After homogenization, aliquots of samples were taken and lyophilized, and then the lyophilized samples were powdered and stored at -20℃ or below. Immediately before the analysis, the samples were re-lyophilized to evaporate moisture absorbed during the powdering and storing process.

Chemical analysis.

Kjeldahl's method [15] was employed for measuring nitrogen amounts. Protein amounts were calculated by multiplying measured nitrogen values by 6.25 [17]. For the analysis of fat amounts, Soxhlet's ether-extraction method [16] was employed. Carbohydrate amounts were calculated as differences between the amounts of subtracted protein, fat, and ash and the total dried sample weights [9, 17]. Calculations of ash amounts were based on residual sample weights after the autoanalyzer measurements [18].

Measurement by the autoanalyzer and reproducibility of results.

In this study, a carbon, hydrogen, and nitrogen autoanalyzer (CHN analyzer Model MT-2; Yanagimoto, Kyoto) was used to measure the elemental compositions of food samples. Approximately 2 mg of dried sample was burnt out completely at 850 to 900℃ in a mixed flow of He gas (150 ml/min) and pure oxygen gas (8 ml/min) for conversions of elements into H_2O, CO_2, and N_2 gases [18]. The elemental compositions of carbon, hydrogen, and nitrogen were calculated automatically from the proportions of these gases detected by thermal conductivities [18]. The oxygen composition of the sample was estimated as a residual value by the subtraction of carbon, hydrogen, nitrogen, and ash compositions. The autoanalyzer measurement procedures were automated and required 7.5 min per sample.

To examine the reproducibility in the measurement of the food samples, 20 samples were randomly selected from the group of lunches. Each homogenized sample was poured into two cups, and each pair of cups was individually lyophilized, stored, and measured by the autoanalyzer.

Comparison of food-sample nitrogen amounts determined by Kjeldahl's and the autoanalyzer methods.

For the comparison of nitrogen amounts of the food samples, 20 samples were selected from the group of dishes. Nitrogen measurements by both methods were carried out in a double-blind manner. For Kjeldahl's method [15], approximately 2.0 g of dried sample was applied, while roughly 2.0 mg of sample was used for the autoanalyzer method.

Standardization of the elemental compositions of three major nutrients.

Proteins generally consisted of 50 to 55% carbon, 6 to 8% hydrogen, 20 to 30% oxygen, 15 to 18% nitrogen, and 0 to 4% sulfur. The nitrogen composition is the theoretical base of Kjeldahl's method to obtain the protein content by multiplying with the factor 6.25 (100/16) the nitrogen amount [17]. According to previous reports using this method, the standardized elemental composition of protein is assumed to be 53.0% carbon, 7.0% hydrogen, 22.0% oxygen, 16.0% nitrogen, and 2.0% sulfur.

Elemental compositions of some fatty acids are summarized in Table 1. Compositions of fatty acids vary among foodstuffs. For the estimation of the fat proportion in a single raw foodstuff, it may be possible to use the specific elemental composition of fatty acids in that foodstuff. To estimate the fat proportions in cooked foods, it is necessary to introduce a widely applicable elemental composition for fat. For this purpose, palmitic and oleic acids, which are major fatty acids of many foodstuffs [19], were selected as representatives of saturated and unsaturated fatty acids, respectively. For the standardized elemental composition of fat, the following mean values of palmitic and oleic acids were used: 75.7% carbon, 12.4% hydrogen, and 11.9% oxygen.

Carbohydrates were represented in general by the equation $Cn(H_2O)m$. Small variations in elemental compositions of carbohydrates were caused by differences of assembled sugars and chemical bindings. Therefore, amylose was chosen as the standard carbohydrate, and the standardized elemental composition of carbohydrate is assumed to be 44.5% carbon, 6.2% hydrogen, and 49.3% oxygen.

Estimation of nutrient amounts using the standardized elemental compositions of nutrients.

According to the standardized elemental compositions described above, nutrient proportions in food samples could be obtained from the following 5 simultaneous equations:

$$S_{C\%} = 53.0\times P + 75.7\times F + 44.5\times C \quad \text{..... (eq. 1)}$$
$$S_{H\%} = 7.0\times P + 12.4\times F + 6.2\times C \quad \text{..... (eq. 2)}$$
$$S_{O\%} = 22.0\times P + 11.9\times F + 49.3\times C \quad \text{..... (eq. 3)}$$
$$S_{N\%} = 16.0\times P \quad \text{..... (eq. 4)}$$
$$1.00 = P + F + C + A, \quad \text{..... (eq. 5)}$$

where $S_{C\%}$, $S_{H\%}$, $S_{O\%}$, and $S_{N\%}$ are the carbon, hydrogen, nitrogen, and oxygen compositions of the food sample, respectively, and P, F, C, and A are the proportions of protein, fat, carbohydrate, and ash in the food sample, respectively.

Estimation of nutrient amounts using a new factor.

To reduce the number of simultaneous equations, a new factor was introduced. This factor, the food coefficient (FC), basically represents the ratio of oxygen requirement and carbon dioxide release for combustion of foods under considering metabolic paths. This new factor (FC) is basically identical to the respiratory quotient (RQ), which has traditionally been used in physical metabolic studies [20]. The following two assumptions were used for making an equation of FC: 1) carbon, hydrogen, and nitrogen were assumed to be converted into carbon dioxide, water, and urea, respectively; and 2) internally existing oxygen was assumed to be preferentially used for conversions. The new factor (FC) was represented as

$$FC = CO_2 / O_2$$
$$= (S_{C\%} - S_{N\%}\times[C_A / N_A\times 0.5]) / (S_{C\%} - S_{N\%}\times[C_A / N_A\times 0.75] + S_{H\%}\times[C_A / H_A\times 0.25] - S_{O\%}\times[C_A / O_A\times 0.5]), \quad \text{..... (eq. 6)}$$

where C_A, H_A, N_A, and O_A are the atomic weights of carbon (12.011), hydrogen (1.008), nitrogen (14.0067), and oxygen (15.999), respectively.

The FC value of protein, fat, and carbohydrate was calculated as 0.834, 0.701, and 1.000, respectively, using each standardized elemental composition. Each nutrient proportion in the food sample was obtained from the following 3 simultaneous equations:

$$S_{FC} = 0.834\times P + 0.701\times F + 1.000\times C \quad \text{..... (eq. 7)}$$
$$S_{N\%} = 16.0\times P \quad \text{..... (eq. 4)}$$

$1.00 = P+F+C+A,$ ..... (eq. 5)

where S_{FC} is a FC value of the food sample.

Statistical analysis.

Statistical analysis was performed on a Macintosh IIsi using StatView software. The mean and standard deviation of the differences between the measurements were calculated to obtain a repeatability coefficient or limits of agreement [21, 22]. Regression analysis was carried out to examine the correlations between each nutrient proportion obtained by the chemical and new estimation methods. Statistically significant differences between the chemical and estimated values were determined by paired t-test. P values of less than 0.05 were considered significant.

Table 1 Elemental compositions of fatty acids.

Fatty Acids		Carbon (%)	Hydrogen (%)	Oxygen (%)	Food Coefficient
Saturated					
Lauric	(C12)	71.95	12.08	15.97	0.706
Myristic	(C14)	73.63	12.36	14.01	0.700
Palmitic	(C16)	74.94	12.58	12.48	0.696
Stearic	(C18)	76.00	12.76	11.25	0.692
Arachidic	(C20)	76.86	12.90	10.24	0.690
Unsaturated					
Oleic	(C18:1)	76.54	12.13	11.33	0.706
Linoleic	(C18:2)	77.09	11.50	11.41	0.720
Linolenic	(C18:3)	77.65	10.86	11.49	0.735
Arachidonic	(C20:4)	78.90	10.59	10.51	0.741

Carbon, hydrogen, and oxygen values are represented by weight percentages.

The food coefficient was calculated by eq. 6 as follows.

$$FC = (S_{C\%} - S_{N\%} \times [C_A / N_A \times 0.5]) / (S_{C\%} - S_{N\%} \times [C_A / N_A \times 0.75] + S_{H\%} \times [C_A / H_A \times 0.25] - S_{O\%} \times [C_A / O_A \times 0.5]), \quad \text{..... (eq. 6)}$$

where $S_{C\%}$, $S_{H\%}$, $S_{O\%}$, and $S_{N\%}$ are the carbon, hydrogen, nitrogen, and oxygen compositions of the food sample, respectively, and C_A, H_A, N_A, and O_A are the atomic weights of carbon (12.011), hydrogen (1.008), nitrogen (14.0067), and oxygen (15.999), respectively.

Results

Reproducibility of food-sample measurements.

Measurements were carried out twice in a double-blind manner using approximately 2.0 mg of sample for each measurement (A and B, Table 2). Mean levels of the ash proportion of A and B were 1.45% and 1.42%, respectively. To examine the reproducibility of these results, a ratio of the measurements, A/B (%), was calculated (Table 2). Very close values were observed between each corresponding pair of measurements, with the A/B values being close to 100 (%) in all cases. The A/B values of nitrogen had a larger standard deviation (SD) and coefficient of variation (CV) than the other elements, but the CV was still less than 5%. The SD and CV of the A/B values of carbon, hydrogen, and oxygen were very small. No significant differences in any of the elements between measurements A and B were observed by paired t-test. Furthermore, the mean and standard deviation of the differences between the measurements were calculated to obtain a repeatability coefficient adopted by the British Standards Institution [21, 22]. The repeatability coefficients of carbon, hydrogen, nitrogen, and oxygen were 0.52%, 0.08%, 0.28%, and 0.72%, respectively.

Comparison of food-sample nitrogen amounts determined by Kjeldahl's and the autoanalyzer methods.

As shown in Table 3, nitrogen amounts of 20 samples measured by both methods showed wide and nearly identical ranges. The mean and standard deviation of the differences between the measurements were calculated to determine a measuring agreement [21, 22]. The mean difference was -0.07% with a 95% confidence interval of -0.13% to -0.01%. The lower and upper limits of agreement were -0.31% and 0.18% with 95% confidence intervals of -0.42% to -0.21% and 0.08% to 0.28%,

Table 2 Reproducibility of autoanalyzer measurements

Sample No.	Carbon (%)			Hydrogen (%)			Nitrogen (%)			Oxygen (%)		
	(A)	(B)	(A/B)	(A)	(B)	(A/B)	(A)	(B)	(A/B)	(A)	(B)	(A/B)
1	49.45	49.41	100.1	7.46	7.44	100.3	2.92	3.09	94.5	38.54	38.46	100.2
2	47.36	47.61	99.5	7.06	7.05	100.1	3.38	3.54	95.5	40.32	39.91	101.0
3	50.21	49.83	100.8	7.47	7.46	100.1	3.47	3.25	106.8	37.33	38.04	98.1
4	45.87	45.96	99.8	6.84	6.81	100.4	2.71	2.68	101.1	43.38	43.29	100.2
5	44.78	44.84	99.9	6.59	6.62	99.5	2.20	2.18	100.9	45.40	45.42	99.9
6	46.55	46.03	101.1	6.84	6.79	100.7	3.21	3.07	104.6	41.73	42.26	98.7
7	45.65	45.83	99.6	6.79	6.74	100.7	4.25	4.28	99.3	41.58	41.24	100.8
8	46.60	46.62	100.0	6.92	6.86	100.9	3.22	3.26	98.8	41.84	41.85	100.0
9	47.17	47.01	100.3	6.99	6.91	101.2	2.88	2.69	107.1	41.37	42.08	98.3
10	46.21	46.40	99.6	6.83	6.83	100.0	2.95	2.92	101.0	42.61	42.49	100.3
11	47.28	47.05	100.5	6.96	7.03	99.0	2.37	2.30	103.1	42.49	42.76	99.4
12	49.80	49.92	99.8	7.38	7.41	99.7	3.30	3.47	95.1	37.89	37.85	100.1
13	49.02	48.41	101.3	7.28	7.28	100.0	2.94	2.97	99.0	39.31	39.91	98.5
14	48.77	48.91	99.7	7.20	7.23	99.5	2.94	2.95	99.6	39.70	39.38	100.8
15	45.61	45.50	100.2	6.67	6.62	100.7	3.13	3.24	96.6	42.87	43.09	99.5
16	48.25	48.57	99.3	7.21	7.23	99.7	2.99	3.05	98.0	39.87	39.63	100.6
17	45.57	45.61	99.9	6.71	6.78	98.9	2.23	2.23	100.0	44.30	44.28	100.1
18	45.93	46.01	99.8	6.90	6.86	100.6	3.30	3.18	103.8	42.40	42.53	99.7
19	46.67	46.55	100.3	6.86	6.83	100.4	2.87	2.60	110.4	42.65	42.94	99.3
20	48.75	48.33	100.9	7.28	7.23	100.7	3.94	3.66	107.7	38.45	39.08	98.4
Mean	47.28	47.22	100.1	7.01	7.00	100.2	3.06	3.03	101.1	41.20	41.33	99.7
SD	1.61	1.55	0.5	0.27	0.27	0.6	0.52	0.51	4.5	2.20	2.16	0.9
CV (%)	3.40	3.28	0.5	3.79	3.87	0.6	16.41	16.80	4.4	5.34	5.22	0.9

Mean levels of ash proportion of A and B were 1.45% and 1.42%, respectively.

Oxygen composition (%) was calculated as a residual value by the subtraction of carbon, hydrogen, and ash compositions.

respectively. In addition, a very good linear relation was observed between Kjeldahl's (y) and the autoanalyzer (x) methods (y= 0.995x -0.047; r= 0.999; p< 0.001). Therefore, protein amounts were calculated by multiplying the nitrogen values obtained by the autoanalyzer method by the conversion factor, 6.25.

Nutrient estimation using the standardized elemental composition.

The mean elemental composition of A in Table 2, 47.28% carbon, 7.01% hydrogen, 3.06% nitrogen, 41.20% oxygen, and 1.45% ash, was used for the estimation. The protein proportion was obtained from eq. 4. Each nutrient proportion in the food sample appeared to be obtainable from the remaining equations, eqs. 1, 2, 3, and 5. However, the proportions of fat and carbohydrate, which satisfy the remaining simultaneous equations, could not be obtained. Calculated proportions for fat and carbohydrate fluctuated on intersections of lines derived from the simultaneous equations (Fig. 1).

The estimation method using the new factor.

According to the above results, the new factor (FC) for the mean elemental composition of A in Table 2 could be derived using eq. 6. The FC value was calculated as 0.906 using the mean values above. Using this FC value, the proportions of fat and carbohydrate were calculated by eqs. 7 and 5 as 0.1592 and 0.6351, respectively (shown in Fig. 1 as a closed circle).

In conclusion, a new method for estimating protein, fat, and carbohydrate proportions in cooked foods was established using eq. 6 and the simultaneous equations 4, 5, and 7.

Comparison of the fat proportion derived by the Soxhlet's ether-extraction and new estimation methods.

To compare the fat proportion derived by the Soxhlet's ether-extraction and new estimation methods, the remaining 40 samples for both the individual dish and lunch groups were used.

As shown in Fig. 2, significant correlations between the Soxhlet's ether-extraction and the new estimation methods were observed in the individual dish (upper graph) and lunch (lower graph) groups (p< 0.001 for both).

The mean and standard deviation of the differences between the methods were calculated to determine a measuring agreement [21, 22]. In the individual dish group, the mean difference was -0.025 (g/g) with a 95% confidence interval of -0.048 to -0.003 (g/g). The lower and upper limits of agreement were -0.168 and 0.117 (g/g) with 95% confidence intervals of -0.208 to -0.129 and 0.078 to 0.157 (g/g), respectively. In the lunch group, although the slope of the correlation line was close to 0.5, the mean difference (0.024 [g/g]) between the methods was the same as for the individual dish group. The 95% confidence interval of

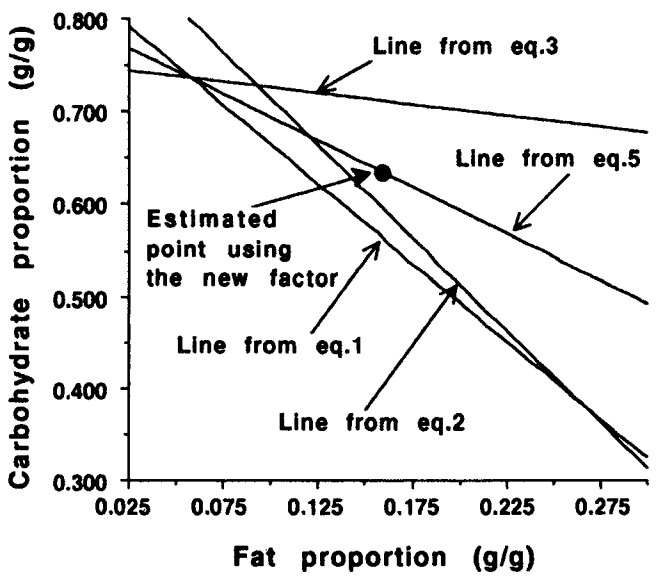

Fig. 1 Estimation of fat and carbohydrate proportions in food samples from the standard elemental compositions.

Individual lines represent eqs. 1, 2, 3, and 5, respectively. The fat (X-axis) and carbohydrate (Y-axis) proportions are indicated by weight of the food samples (g/g).

The fat and carbohydrate proportions estimated by the new method using the food factor, FC, are represented with a closed circle.

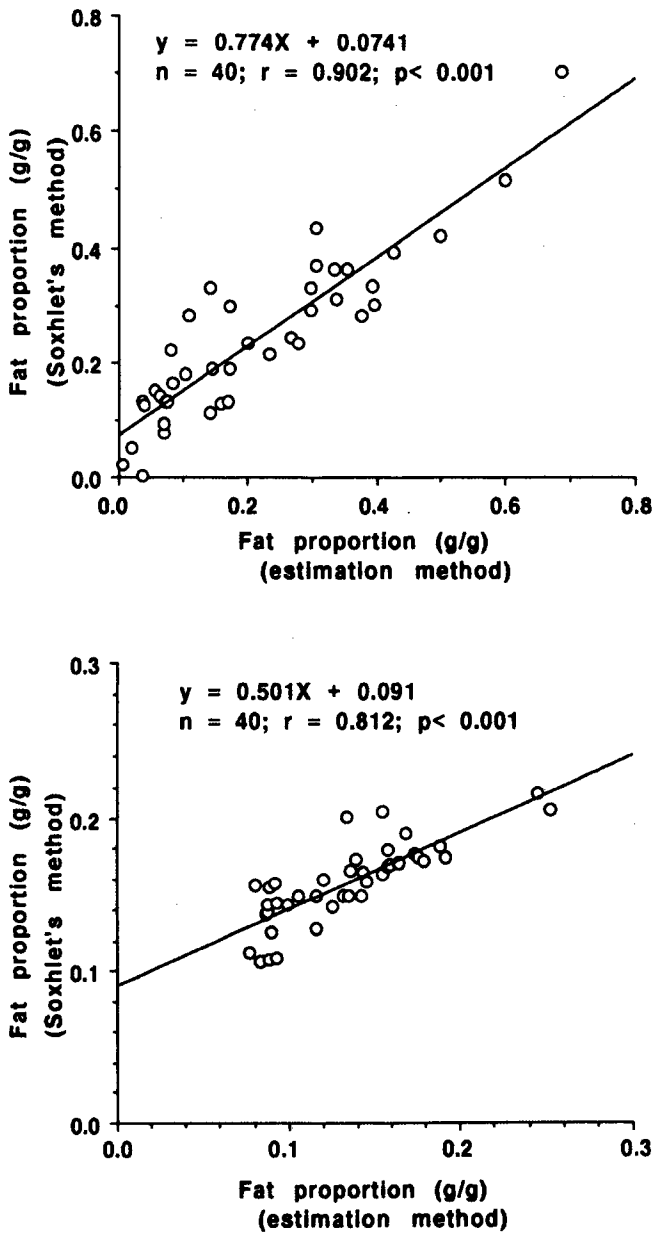

Fig. 2 Correlations of fat proportions in the food samples between the Soxhlet's ether-extraction and new estimation methods.

The fat proportions are indicated by weight of the food samples (g/g). Correlation analysis was carried out in both the individual dish (n = 40, upper) and lunch (n = 40, lower) groups. Significant correlations between the methods were evaluated by coefficients of correlations.

the mean difference was -0.033 to -0.016 (g/g). The lower and upper limits of agreement were -0.077 and 0.029 (g/g) with 95% confidence intervals of -0.092 to -0.062 and 0.014 to 0.043 (g/g), respectively.

These overestimating tendencies were also revealed by the paired t-test in the individual dish and lunch groups, $p< 0.05$ and $p< 0.001$, respectively.

Comparison of the carbohydrate proportion determined by chemical and the new methods.

As described above, the remaining 40 samples in both the individual dish and lunch groups were used for comparing the carbohydrate proportion. Significant correlations of the carbohydrate proportion between the methods were observed in both the individual dish and lunch groups ($p< 0.001$ for both), as shown in Fig. 3. Correlation coefficients in the carbohydrate proportions were better than in the cases of fats, and the slopes of the correlation lines were close to 1.0 in both groups.

The mean and standard deviation of the differences between the methods were calculated to determine a measuring agreement[21,22]. In the individual dish group, the mean difference was 0.021 (g/g) with a 95% confidence interval of -0.001 to 0.043 (g/g). The lower and upper limits of agreement were -0.117 and 0.158 (g/g) with 95% confidence intervals of -0.155 to -0.079 and 0.120 to 0.197 (g/g), respectively. On the other hand, in the lunch group, the mean difference was 0.015 (g/g) with a 95% confidence interval of 0.007 to 0.023 (g/g). The lower and upper limits of agreement were -0.036 and 0.066 (g/g) with 95% confidence intervals of -0.050 to -0.022 and 0.052 to 0.080 (g/g), respectively.

No significant difference was observed among samples of the individual dish group by paired t-test. On the other hand, in the lunch group, a significant difference ($p< 0.001$) was observed in the carbohydrate proportion between the methods.

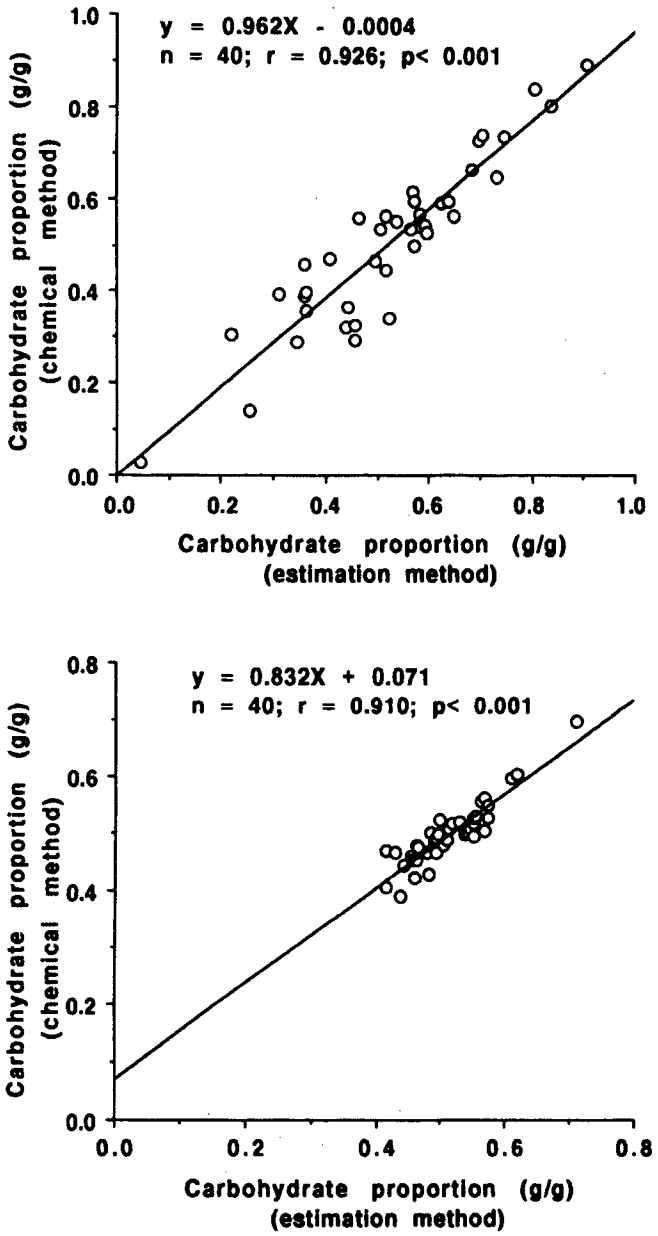

Fig. 3 Correlations of carbohydrate proportions in the food samples between the chemical and the new estimation methods.

The carbohydrate proportions are indicated by weight of the food samples (g/g). For further details see Figure 2.

Discussion

In this study, a rapid, simple, and reliable method of estimating major nutrients in cooked foods was developed using a carbon, hydrogen, and nitrogen autoanalyzer. This use of an autoanalyzer provides important information for determining the molecular formulae of organic compounds. Unlike chemicals, however, food samples are not single pure substances, and this lack of uniformity introduces and amplifies measurement errors that reduce the reproducibility of autoanalyzer measurements. To evaluate the reproducibility of food sample measurements, food samples were randomly selected from a group of lunches. It was thought that it would be more difficult to establish uniformity among those lunch-group samples because they consisted of more types and volumes of foodstuffs than the samples of the individual dish group. According to the results shown in Table 2, however, the autoanalyzer measurements were not influenced by the use of small amounts of nonuniform samples. The results also showed good reproducibility. Therefore, the results proved that the carbon, hydrogen, and nitrogen autoanalyzer was applicable for measurements of cooked foods.

Protein amounts in foodstuffs should be analyzed by suitable analytical methods according to their particular characteristics. However, the application of specific analytical methods to the measurement of cooked foods is problematic due to complications caused by the individual characteristics of the foodstuffs and of their changes during the culinary process. For this reason, the nitrogen amounts analyzed here were multiplied by a conversion factor (6.25)[17] for the estimation of protein proportions in cooked foods. Kjeldahl's method is most reliable for these purposes, and is widely used to analyze nitrogen amounts in food samples[15]. As shown in Table 3, the wide-range results of the autoanalyzer method were nearly identical to those obtained using Kjeldahl's method. The mean difference (-0.07%) and the limits of agreement (-0.31% and 0.18%) were small enough and a significant correlation was observed between the two methods ($p< 0.001$). Therefore, nitrogen amounts measured by the autoanalyzer method were used for the estimation of the protein proportions in cooked foods.

The three major nutrient proportions in food samples appeared to be obtainable from the standardized elemental compositions using the simultaneous equations 1 to 5. As shown in Fig. 1, the proportions of fat and carbohydrate fluctuated on

Table 3 Comparison of the nitrogen amounts (N%) measured by the autoanalyzer and Kjeldahl's methods.

No.	Name of Food Samples	N% measured by Autoanalyzer	Kjeldahl's	ratio (%)
1.	Cooked Rice A	1.28	1.28	100.1
2.	Cooked Rice B	1.28	1.27	100.8
3.	White Bread A	2.27	2.17	104.6
4.	White Bread B	2.34	2.27	103.1
5.	Bread Type Rolls	2.13	1.96	108.7
6.	French Bread	2.19	1.98	110.6
7.	Boiled Eggplant	1.71	1.64	104.0
8.	Tempura (Okura)	2.33	2.37	98.3
9.	Croquettes	3.28	3.22	101.9
10.	Curry	3.26	3.23	100.9
11.	Tempura (Fish Paste)	4.63	4.55	101.8
12.	Salad	4.39	4.45	98.5
13.	Processed Cheese	5.65	5.19	108.9
14.	Fried Sausage	5.00	5.04	99.3
15.	Scrambled Egg	6.09	6.11	99.6
16.	Spaghetti (meat sauce)	3.24	3.25	99.8
17.	Pork and Beans	6.53	6.41	101.8
18.	Pork Loin Ham	8.02	7.84	102.3
19.	Fried Chicken	6.22	6.31	98.6
20.	Boiled Chicken	10.66	10.60	100.5

ratio (%): calculated as Autoanalyzer / Kjeldahl's method × 100

intersections of lines derived from the simultaneous equations. A new factor, the food coefficient (FC), was introduced to reduce the number of simultaneous equations, and this new factor solved the problem mentioned above. In conclusion, using the new factor, FC, and the simultaneous equations 4, 5, and 7, a new method of estimating protein, fat, and carbohydrate proportions in cooked foods was established.

The fat proportion obtained by the new estimation method was significantly correlated with that of Soxhlet's ether-extraction method (shown in Fig. 2). The mean differences and the limits of agreements for the individual dish and lunch groups were small enough. However, the new estimation method showed an overestimating tendency. Significant overestimations were revealed by the paired t-test in both the individual dish ($p < 0.05$) and lunch ($p < 0.001$) groups. These overestimations were thought to be caused by ineffective extraction of fats by Soxhlet's method [16], since it is known that this method does not fully extract fats in foods [23]. Another possible explanation was that the FC value of fat used in this estimation method may have been a little larger than the actual fatty acids composition. For estimating nutrients in a single foodstuff, it may be advantageous to use a FC value of fat that suitably represents the actual fatty acids composition.

Very good correlations of carbohydrate proportion (shown in Fig. 3) and good measuring agreements for the individual dish and lunch groups were observed between the chemical and new estimation methods. These results only reflected the difference of fat proportion. One reason for the better correlations in the carbohydrate proportion may be that the proportion of carbohydrate was relatively dominant and little affected by the small difference in the fat proportion.

According to the results, the three major nutrient proportions in cooked food samples estimated by the new method correlated to those determined analytically. This new method, which required only 7.5 min per sample to simultaneously estimate three major nutrients, may potentially reduce the cost and time and the logistical and personnel constraints associated with nutrition surveys [6, 10, 11]. Ulijaszek [5] indicated that large samples are needed to obtain nutrient intakes representative of a group if the 24-hour recall method is used, while smaller samples are acceptable if weighed dietary intakes for a number of days are used. In other words, higher accuracy requires the use of smaller samples. Although the food frequency questionnaire method is easily and quickly completed by subjects and easy for researchers to code and analyze [4], an increase in the number of days entails higher costs, and this could limit the practicability of the study [24].

The new estimation method established in this study may be preferentially applicable to mixed and cooked foods, including unknown foodstuffs and the preparation, trim, cooking, or brand methods used where the agreement was lower than by any of the present survey methods. Again, this new method is based on the duplicate portion sampling technique, which provides the most accurate information [12, 13]. Weaknesses in methodology at the point of data collection cannot be overcome by expansion of nutrient databases, increased precision of nutrient values, or sophisticated statistical analysis [10]. Data collection techniques should be simple, and not excessively time-consuming [10] or troublesome for the participants [8].

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