FOOD COMPOSITION

Conductometric and Colorimetric Determination of Volatile Acidity of Vinegars by Flow-Injection Analysis

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Recent methods for determination of the volatile acidity of vinegars are relatively slow (about 40 min) and involve techniques subject to a variety of errors (ca 2.5%). The present paper describes a method that provides results in a shorter time (ca 2 min, including dilution), with a smaller relative error rate (ca 1%). Conductometric analysis consists of the injection of the sample in a deionized water stream that then flows past a PTFE membrane separator. Acetic acid diffuses through the membrane to another deionized water stream that passes through a conductivity cell. Colorimetric analysis also consists of sample injection into a deionized water stream that passes through the same PTFE membrane separator. However, the acetic acid diffuses into a bromocresol purple solution stream at pH 7. This solution passes through a flow cell in a spectrophotometer set at 540 nm. Before injection, samples were treated with hydrogen peroxide to ensure complete oxidation of sulfite to sulfate. Results of the proposed method were also compared with another similar method. At a 95% confidence level, the statistical *t*-test indicates no significant difference between them. Typical estimates of the relative standard deviations obtained with the new methods are ca 1%. Analyses were performed with red and white wine vinegars.

Since its introduction in 1974–1975 in a classic paper (1, 2), flow-injection analysis (FIA) has been a valuable means of automating analyses and increasing sample output in most analytical laboratories. Besides being a method that permits many analyses in a short period of time, for analysis of volatile acidity of vinegars in particular, FIA systems provide a substantial increase in the precision of results when compared with traditional methods. Generally, those methods have many steps, which increases the rate of error.

Baadenhuijsen and Seuren-Jacobs (3) used gas diffusion in FIA in a determination of carbon dioxide in plasma using a gas-permeable membrane. Gas-permeable membranes in FIA systems are now widely used to transfer certain compounds from a donor (sample) stream to an acceptor (detector) stream. The membrane transport process in a flow-through unit and its dependence on characteristic membrane parameters were investigated by Van der Linden (4), both from the theoretical and the practical points of view. Some volatile compounds (e.g., carbon dioxide, ammonia, and acetic acid) have been studied using different types of gas-diffusion membranes.

Gas diffusion is a very selective technique because few species are sufficiently volatile at room temperature. Some compounds (e.g., carbon dioxide, sulfur dioxide, HCN, HF, HCl, and acetic acid) can be measured by this technique, depending on the pH of the donor stream. It is also interesting to note that these species will rarely be present in the sample at the same time. However, some samples, such as beverages, present high amounts of carbon dioxide, which can interfere in the determination of relatively small amounts of sulfur dioxide. In such cases, a more selective, colored reagent is preferable to an acid–base indicator.

In the analysis of volatile acidity of vinegars, the only species in the sample that will permeate the microporous Teflon® membrane are acetic acid, carbon dioxide, and sulfur dioxide. Carbon dioxide does not interfere because of the low pH of the sample; sulfur dioxide can be eliminated by the use of hydrogen peroxide.

Vinegar is a product in which 100 g contains 5 to 15.5 g anhydrous acetic acid produced by acetic fermentation of liquids containing alcohol (5). Vinegar is mainly used by consumers for acidification of salads and vegetables and for seasoning meat and fish; the food industry uses vinegar to preserve and season food at the same time. Nunheimer and Fabian (6) studied the relationship between dissociation constants of several acids and the inhibition of microorganisms in foodstuffs. They found that, when compared with citric acid, lactic acid, malic acid, and tartaric acid, acetic acid is a stronger growth inhibitor of microorganisms at a higher pH than other acids. Szakall (7) found that vinegar has a specific inhibitory effect on the growth of microorganisms, as compared to diluted acetic acid, whose effect is a function of acid concentration only. The reason for this phenomenon is not yet known.

Besides acetic acid and alcohol, vinegar contains secondary constituents that contribute to its smell, taste, and preserving qualities. These constituents have their origin in the raw material, in added nutrients, and in the water used for dilution.

Vinegar can be analyzed for 2 different reasons: (*a*) for process control using routine methods, and (*b*) for a comprehensive knowledge of its chemical constituents using special methods. In vinegars, it is most important to measure volatile acidity, fixed acidity, and total acidity.

METHOD

Samples are treated separately with a few drops of hydrogen peroxide and then analyzed in a flow-injection system. The diffused acetic acid changes the conductivity [in microsiemens (μS)] of a deionized water stream (conductometric method) or the color of the bromocresol purple (BCP) indicator solution stream (colorimetric method). Absorbances are read in a 10 mm flow cell at 540 nm.

Apparatus

(**a**) *Peristaltic pump.*—Ismatec GJ04 mp 13 at a flow rate of 1.26 mL/min.

(**b**) *Sampling valve system.*—Microvolume 2-position sampling valve fabricated in our laboratory, made of graphitic Teflon.

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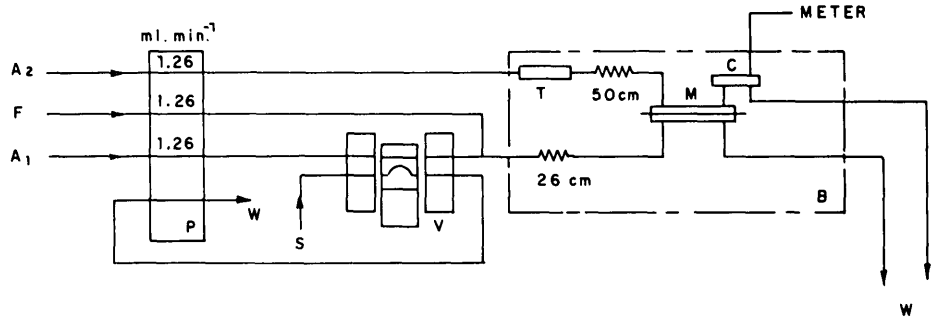

Figure 1. Conductometric flow-injection manifold. T = ion-exchange resin column, P = peristaltic pump, S = sample inlet, V = sampling valve system, B = water bath, M = diffusion cell, C = conductance flow cell, W = waste, A_1 and A_2 = deionized water streams, and F = 0.05M sulfuric acid solution.

(c) Diffusion cell.—Gas-diffusion unit similar to models described in Refs. 4 and 8. Each block, made of acrylic resin, had a shallow groove 0.1 mm deep, 3 mm wide, and 5 cm long. Commercial PTFE (Teflon) microporous tape was placed between the 2 pieces; the unit was secured with 6 screws.

(d) *Conductance flow cell.*—Stainless-steel flow cell (as described in Ref. 8) for conductance measurement. Estimated volume is 60 μL. The cell was covered with epoxy resin to isolate it from the water bath in which it was immersed.

(e) *Conductivity meter.*—Micronal, model B331 connected to a chart recorder.

(f) *Spectrophotometer.*—Zeiss PM2D, equipped with 10 mm flow cell (volume 50 μL) connected to a chart recorder.

Reagents

Prepare all reagents from analytical reagent quality chemicals unless otherwise specified.

(a) *Acetic acid standards.*—Concentrated acetic acid diluted with boiled deionized water to produce solutions 0.2 to 0.6% in acetic acid.

(b) *Bromocresol purple (BCP) solution.*—(1×10^4M). Dissolve 0.27 g BCP in 10 mL ethanol; complete volume to 500 mL with boiled deionized water. Take 50 mL of this solution and dilute with boiled deionized water to 500 mL to produce working solution.

Analytical System

Schematic flow diagrams for conductometric and colorimetric determinations of volatile acidity of vinegars are shown in Figures 1 and 2.

Conductometric analysis.—Combine injected sample (S), previously treated with hydrogen peroxide, with deionized water carrier stream (A_1) pumped at a flow rate of 1.26 mL/min. Add 0.05M sulfuric acid solution (F) and mix in a 26 cm long coil. After mixing, acetic acid diffuses through the Teflon membrane separator (M) to another deionized water stream (A_2) that passes through the conductivity cell. This deionized water stream (A_2) passes, initially, through a column containing an ion-exchange resin to guarantee water free of ions. Immerse conductivity cell, diffusion cell, and resin column in a constant-temperature water bath to avoid temperature changes during analysis.

Colorimetric analysis.—Combine injected sample (S), treated in the same way, with deionized water stream (A_1) pumped at a flow rate of 1.26 mL/min. After injection, acetic acid diffuses through the Teflon membrane separator (M) to the bromocresol purple solution stream (I) at pH 7, passing through a flow cell in the spectrophotometer with absorbance measured at a wavelength of 540 nm. To avoid CO_2 interference (from atmosphere), flask with BCP solution should be protected with a tube containing solid $CaCl_2/NaOH/CaCl_2$.

Preparation of Samples

Add 3 to 5 drops 3.5M hydrogen peroxide to 10 mL vinegar in a 100 mL volumetric flask and complete volume with boiled deionized water. Solutions will contain ca 0.38 to 0.48 g acetic acid/100 mL solution.

Results and Discussion

Tables 1 and 2 show results obtained with FIA methods and with the Jaulmes method (9), which is similar to the AOAC method (10). To statistically compare results, the Student's *t*-test was used (11).

Calibration curves are not linear (Figures 3 and 4); however, volatile acidity can be evaluated by graphical interpolation with acceptable precision. Examination of calibration data show that the experimental curve fits the equation $y = C + Bx + Ax^2$, where y = peak height, x = volatile acidity and A, B, and C = adjustable parameters. Student's *t*-test values in Tables 1 and 2 show that there is no statistical difference between results at a 95% confidence level.

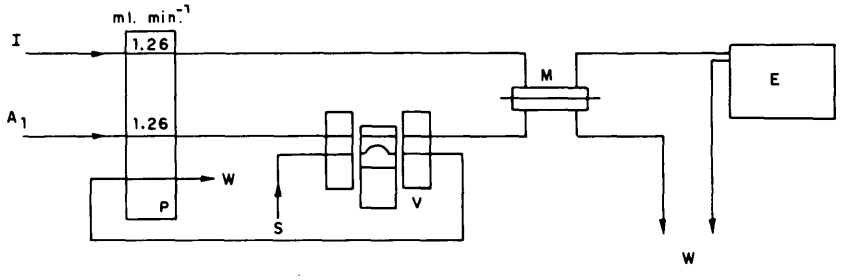

Figure 2. Colorimetric flow-injection manifold. P = peristaltic pump, S = sample inlet, V = sampling valve system, M = diffusion cell, E = spectrophotometer, W = waste, A_1 = deionized water stream, I = BCP solution stream.

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Table 1. Volatile acidity of wine vinegars (g acetic acid/100 mL vinegar) using conductometric FIA system and Jaulmes method (9) (%)

Sample	FIA[a]	t_d	FIA[b]	t_e	Jaulmes
1(R)	4.25	0.32	—	—	4.23
2(W)	4.41	0.16	4.41	0.16	4.40
3(R)	4.31	0.47	4.31	0.47	4.34
4(W)	4.52	0.00	4.57	0.79	4.52
5(R)	4.46	1.11	4.62	2.21	4.53
6(R)	4.46	1.90	4.67	1.42	4.58

[a] FIA method (Figure 3) with Teflon sampling loop, 0.9 mm id; volume, 62 μL.

[b] FIA method with polyethylene sampling loop, 0.8 mm id; volume, 120 μL.

Note: (R) = red wine vinegar, (W) = white wine vinegar. Estimates of standard deviations are ±0.04 for FIA method and ±0.08 for Jaulmes. t_d and t_e are calculated Student t values; tabulated t value for the degree of freedom (ν) 4 is 2.776 ($\alpha = 0.05$); $\nu = n_1 + n_2 - 2$ and $n_1 = n_2 = 3$ in this case.

Table 2. Volatile acidity of wine vinegars (g acetic acid/100 mL vinegar) using colorimetric FIA system and Jaulmes method (9) (%)

Sample	FIA[a]	t_d	FIA[b]	t_c	Jaulmes
1(R)	4.20	0.47	4.22	0.16	4.23
2(W)	4.44	0.63	4.43	0.47	4.40
3(R)	4.31	0.47	4.33	0.16	4.34
4(W)	4.52	0.00	4.53	0.16	4.52
5(R)	4.52	0.16	4.52	0.16	4.53
6(R)	4.57	0.16	4.55	0.47	4.58

[a] FIA method (Figure 4) with polyethylene sampling loop, 1.6 mm id; volume, 240 μL.

[b] FIA method with tygon sampling loop, 1.14 mm id; volume, 46 μL.

Note: (R) = red wine vinegar, (W) = white wine vinegar. Estimates of standard deviations are ±0.04 for FIA method and ±0.08 for Jaulmes. t_d and t_e are calculated Student t values; tabulated t value for the degree of freedom (ν) 4 is 2.776 ($\alpha = 0.05$); $\nu = n_1 + n_2 - 2$ and $n_1 = n_2 = 3$ in this case.

Loops

We tested many loops of different materials, volumes, and diameters to try to resolve a problem of retention of acetic acid in the walls of the loop. This phenomenon was responsible for the increase of peak height in the descending curve. It was established, empirically, that either a polyethylene loop (volume 120 μL, 0.8 mm id) or a Teflon loop (volume 62 μL, 0.9 mm id) can be used in the conductometric method without interference from this phenomenon.

For the colorimetric method, a polyethylene loop (volume 240 μL, 1.6 mm id) and a tygon loop (volume 46 μL, 1.14 mm id) presented the best results.

Figure 5 shows results from a faulty loop. For standards of the same acid concentration, the signal was greater for the decreasing order of injections.

The nonlinearity of the calibration curves is probably the result of different factors. In the conductometric method, because acetic acid is a weak electrolyte, the relationship between conductivity and concentration is not linear. A similar effect occurs in the colorimetric method that is within the limits of the validity of Beer's law. In both cases, the relationship between the diffusion rate of acetic acid through the membrane and concentration is not linear.

Because a complete study of the materials, sizes, and diameters of the loops would be an exhaustive work, an empirical selection was made. However, special attention must be paid to the choice of the size and material of the loops in initiating either method.

Comparison with Other Methods

Traditional methods, including those of Jaulmes and Cazenave-Ferré, have 2 principal steps: distillation and titration. In general, the sampling rate is 1 sample/h. According to data obtained from vinegar manufacturers, a relative error rate of 2.5% is considered acceptable.

The advantages of FIA are evident if one compares the sampling rate (60 samples/h) and the relative error rate (1.0%) of the proposed methods with those of traditional

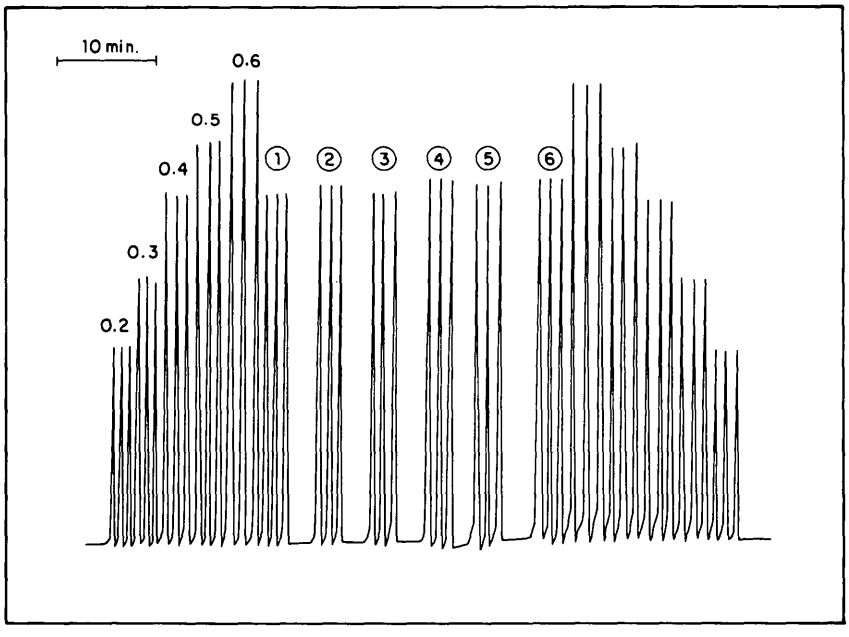

Figure 3. Calibration and sample runs for volatile acidity determination (conductometric system). Left to right: triplicate signals for acetic acid standards (0.2, 0.3, 0.4, 0.5, and 0.6% g acetic acid/100 mL solution). Triplicate signals for vinegars and standards in reverse order. Teflon loop (62 μL, 0.9 mm id).

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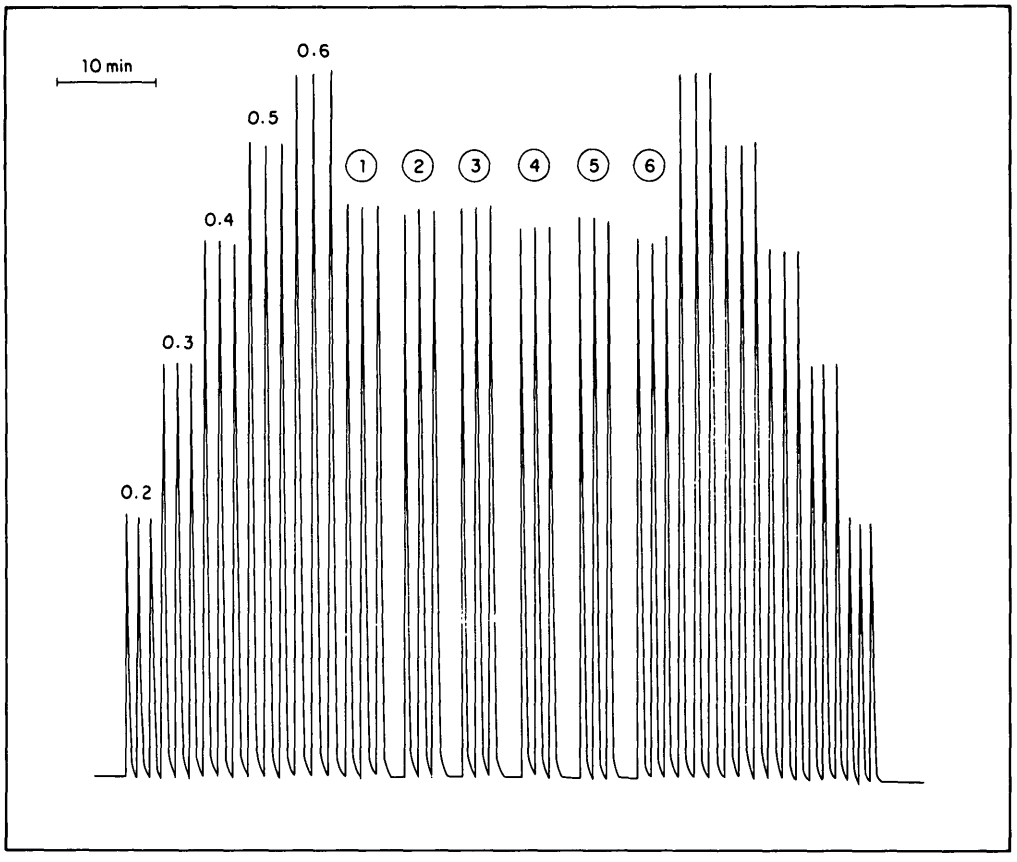

Figure 4. Calibration and sample runs for volatile acidity determination (colorimetric system). Left to right: triplicate signals for acetic acid standards (0.2, 0.3, 0.4, 0.5, and 0.6% g acetic acid/100 mL solution). Triplicate signals for vinegars and standards in reverse order. Polyethylene loop (240 μL; 1.6 mm id).

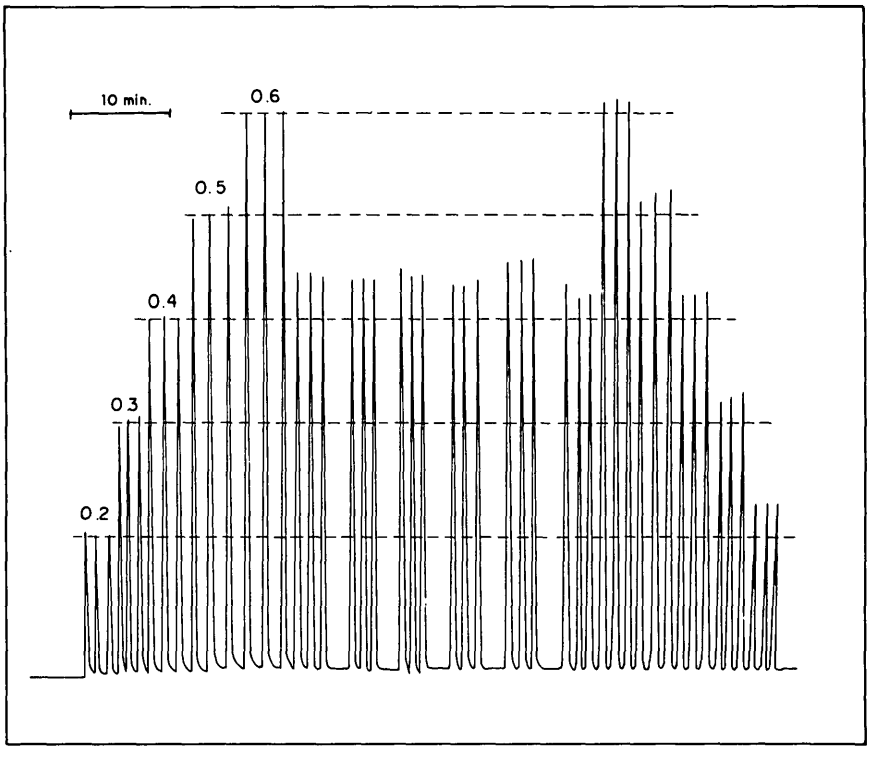

Figure 5. Runs for volatile acidity determination using faulty loop in the colorimetric system (conductometric method, with faulty loop, presents similar behavior). Left to right: triplicate signals for acetic acid standards (0.2, 0.3, 0.4, 0.5, and 0.6% g acetic acid/100 mL solution). Triplicate signals for 6 vinegars; triplicate signals for standards in reverse order. Polyethylene loop (27.5 μL; 0.9 mm, id).

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methods. Although the instrumentation for the traditional methods is less expensive than that for FIA, the additional cost is not great and can easily be justified by the faster sampling rate.

In the present study, analyses were carried out in an apparatus with a steam-boiler, which permitted a sampling rate of 4 samples/h. However, the apparatus that enabled standard deviations similar to those obtained with FIA methods is much more expensive than the FIA systems proposed.

A comparison between the 2 FIA methods proposed shows no significant difference in the precision of the results. However, the colorimetric method is simpler because it needs no water bath and there is no confluence of strong acid (sulfuric acid). On the other hand, the conductometric method permits a more rapid sampling rate (about 70/h) than the colorimetric (about 40–45/h).

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Determination of Total Dietary Fiber in Japanese Foods

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Total dietary fiber was determined in Japanese foods by the Prosky-AOAC method. To accomplish the analyses of unsuitable samples, we introduced a few minor modifications to the versions for (i) seaweed and fruits, (ii) cereals, and (iii) fish and meats. These modified methods were used together with the standard method to obtain results with reasonably good relative standard deviation for 231 foods and 21 groups of mixed foods. In this study, dietary fiber was defined so as not to exclude the nondigestible polysaccharide portions of animal foods. A method was proposed which could estimate more accurately the fiber components of animal foods by measuring the "nondigestible protein" of the fiber sample by the Biuret colorimetric method, instead of the Kjeldahl method, to avoid deducting the values for aminopolysaccharides. In Japanese diets, the amount of fiber obtained from animal foods was less than 5% of the total intake of dietary fiber.

The method of determination of total dietary fiber (TDF) by the enzymatic-gravimetric method of Prosky et al. has been adopted officially by AOAC (1, 2). When applied to the analysis of a number of Japanese foods, this method proved to be inadequate, mainly because rice and related cereals were consumed in large amounts as an important source of dietary fiber. Thus, more accurate measurements were desired for the correct estimation of the amount of intake of dietary fiber for Japanese.

On the other hand, the definition by H. C. Trowell in 1985, namely, that dietary fiber contains "the sum of the polysaccharides and lignin which are resistant to the digestion," is generally interpreted to mean dietary fiber of plant origin (3), and to exclude fibrous components of animal origin. During our analyses, we became aware of the fact that Japanese foods include many kinds of fish and shellfish including shrimp, lobsters, crabs, and squid, together with other seafood such as fish paste products and algae. Some of them are considered a good source of chitin. This situation is reflected in the proposed definition by Japanese authors (4, 5) of dietary fiber: "The whole of nondigestible components in the food which is resistant against human digestive enzymes." In

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