

# A Study on the Analytical Methods for Total Dietary Fiber in Fructan-Containing Foods

SHU-CHI TSAI, SHU-FANG LU, WEN-WEN YU, HSIAO-CHEN LIN AND WEIGUANG FU\*

*Analysis Research and Service Center, Food Industry Research & Development Institute, Hsinchu, Taiwan, R.O.C.*

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## ABSTRACT

The fructans, inulin and oligofructose, were known to possess many of the physiologic properties of dietary fiber (DF). However, they were not listed as DF on labels of foods that contained them, because they cannot be completely recovered by the precipitate of AOAC enzymatic-gravimetric method. The main goal of this study was therefore to develop a quantitative method for total DF in fructan-containing foods. Evaluation was carefully done by combining AOAC 985.29 DF method and AOAC 999.03 fructans method. In addition to three commercial fructan products, asparagus and tea drink samples were selected and spiked with fructans to carry out the method validation. The results were all in excellent agreement with the expected values. Also, associated precision and measurement uncertainties were evaluated as well. Recoveries by the proposed method ranged from 97.02% to 101.35%, with coefficients of variation (CVs) of 0.63~1.17%. Total DF content was more accurate for combined method than either from AOAC 985.29 only or the sum of determining values from AOAC 985.29 plus AOAC 999.03.

Key words: fructans, inulin, fructo-oligosaccharides, dietary fiber, analytical method

## INTRODUCTION

The term "dietary fiber" in food was first described by Hipsley in 1953<sup>(1)</sup>, whose definition and scope has been debated and revised by researchers. In the present stage, the measurement of dietary fiber comes from the values of AOAC 985.29<sup>(2)</sup> or AOAC 991.43<sup>(3)</sup>. Both of them are enzymatic-gravimetric methods, and the principle involves removing fats first, followed by treatment with heat-stable  $\alpha$ -amylase, protease and amyloglucosidase to degrade proteins and starch. Next, four volumes of 95% ethanol was added to precipitate soluble dietary fiber. After precipitates were filtered and weighed, total dietary fiber was determined by subtracting the weight of proteins and ashes from the weight of precipitate. The definition of dietary fiber is mostly based on their physiological properties. In 2001, American Association of Cereal Chemists (AACC) presented the newest definition suggesting that the coverage of dietary fiber should include those short-chain polysaccharides that are soluble in 78% ethanol including fructans<sup>(4)</sup>.

Fructans, including inulin and fructo-oligosaccharides (FOS), are commonly present in plants. Fructans can be found in 1/3 of plants sources and 15% of flowering plants. The common structure of fructan is a group of fructoses with  $\beta$  (2 $\rightarrow$ 1) fructosyl-fructose linkage in the company of additional glucose linked with  $\alpha$  (1 $\rightarrow$ 2) bond at the end. Due to the structure configuration,

it is difficult to be degraded by alimentary enzymes or digesting acid presented in gastrointestinal (GI) tract in human. However, fructans can interact with specific bacteria such as *Bifidobacteria* and undergoes fermentation processes in large intestine. In European countries, fructans are considered as foods or food additives. In United States, Food and Drug Administration (FDA) regulates fructans as Generally Recognized as Safe (GRAS) compounds. Fructans pose certain health benefits to humans. For example, they are beneficial to diabetes patients because digestion of fructans would not elevate the glucose and insulin levels in blood. Moreover, fructans are prebiotic, they are valuable for the growth of intestine bacteria that are beneficial to humans<sup>(5)</sup>. Fermented fructans would produce short-chain fatty acids<sup>(6)</sup>, those short-chain fatty acids would lower the pH in intestine, and this would aid the absorption of minerals especially for magnesium and calcium. Researchers have shown the addition of inulin in diet has a beneficial effect on plasma lipids by decreasing hepatic lipogenesis and plasma triacylglycerol concentrations<sup>(7)</sup>, and it might also lower the cholesterol and triacylglycerol levels in blood<sup>(8)</sup>. In addition, consumption of fructans would cause an increase in fecal mass and prevent constipation. Consumption of fructans could also shorten the time spent in GI tract, thus increasing the sensation of satiety which is suitable for people on diet. Lastly, it would cause reduction of the risk of colon cancer<sup>(5)</sup>. Due to the advantages mentioned above, fructans are popular food additives/ingredients

\* Author for correspondence. Tel: +886-3-5223191 ext. 303;  
Fax: +886-3-5214016; E-mail: fwg@firdi.org.tw

in functional food market. Other than the physiological properties of fructans previously mentioned, fructans have other applications in food processing: fructans with low degree polymerization can be used as sweeteners in food processing for lowering the calorie intake in food. Fructans can be used to prevent dehydration of fruit flesh for yogurt products, and as fat substitute in spread and cheese. Moreover, they can enhance taste and texture, act as emulsifier, increase the stability of bread and baking goods by keeping them moist and fresh longer, and also enhance crispness and expansion of extruded products<sup>(9)</sup>.

For fructan-containing foods, the nutrition labeling of dietary fiber has not been unified and is still based on the regulation of individual countries. Some countries like Finland, Norway, New Zealand and Australia consider fructan as part of dietary fiber, and some do not. The measurements of dietary fibers are based on either the combination of enzymatic-gravimetric method and with additional quantification from AOAC 997.08<sup>(10)</sup> or AOAC 999.03<sup>(11-13)</sup>. However, according to the literature, fructan with higher degree of polymerization would precipitate during precipitation step with alcohol in enzymatic-gravimetric method<sup>(14)</sup>. Small amount of fructans would co-precipitate with the rest of the fibers in 985.29 and that would cause the duplicate quantitation<sup>(14,15)</sup> since fructans were also determined in either AOAC 997.08 or AOAC 999.03. In this study, we investigated a new method based on results from both AOAC 985.29 and AOAC 999.03 methods. First, the filtrate was collected after enzymatic-gravimetric method to analyze its fructan concentrations. Fructan content was also evaluated by comparing different total dietary fiber methods in the hope to set up an appropriate analytical method for the total concentration of dietary fiber.

## MATERIALS AND METHODS

### I. Materials

#### (I) Fruits and Vegetables

Onions, asparagus, leeks, garlic sprouts and burdock were purchased in a store in Hsinchu. After washing, samples were homogenized for further analysis.

#### (II) Fructan-Containing Processed Foods

Tea drinks, cereal powders and milk powders were purchased in a store in Hsinchu. It is noted that, two types of tea drinks were used in this research: fructan added tea drinks were chosen for the determination of fructan and total dietary fiber by different analytical methods (designated as Tea drink 1), whilst teas used in validation of accuracy did not contain additional fructans (designated as Tea drink 2).

#### (III) Fructan-Containing Solutions

Solutions were made from samples by SENSUS (Roosendaal, Netherlands). Fructafit CLR (average degree of polymerization 7), Fructafit IQ (average degree of polymerization 12) and Fructafit TEX (average degree of polymerization 25); the solution concentration was 4.36%, 4.56% and 4.86% respectively.

#### (IV) Certified Reference Material (CRM)

CRM 381 rye flours were from Institute for Reference Materials and Measurements (Geel, Belgium).

## II. Chemicals

Standard chemicals for AOAC 985.29 and AOAC 999.03 methods were used. Maleic acid, acetic acid, *p*-hydroxybenzoic acid hydrazide, trisodium citrate, sodium hydroxide, sodium borohydride,  $\alpha$ -amylase (heat stable), protease, amyloglucosidase and celite were purchased from Sigma Chemical (St. Louis, MO, USA). Sucrase,  $\beta$ -amylase, pullulanase, maltase, fructanase, fructose standard solution were purchased from Megazyme (Wicklow, Ireland). Ethanol, sulfuric acid, acetone, sodium phosphate dibasic (anhydrous) and sodium phosphate monobasic monohydrate were purchased from Merck Inc. (Darmstadt, Germany).

## III. Analytical Methods

#### (I) AOAC 985.29

Total Dietary Fibers in Foods, Enzymatic-Gravimetric Method (abbreviated as 985.29).

#### (II) AOAC 999.03

Measurement of Total Fructan in Foods, Enzymatic-Spectro photometric Method (abbreviated as 999.03).

#### (III) Combination of 985.29 and 999.03 Methods (Abbreviated as Combined Method)

In this method, samples were first analyzed by 985.29 and the filtrate from 985.29 was collected and concentrated with the aid of vacuum to remove alcohol and acetone. After concentration, the flask was washed with hot water (> 85°C) several times. Washing solution was collected and quantified by 999.03 analysis. The data from 985.29 and 999.03 was added for the combined method value. For the conveniences of discussion and to avoid confusion in the tables and content, the method and results by 999.03 using filtrate from 985.29 were assigned as 999.03\*.

## IV. Statistical Analysis

The data were calculated by one way ANOVA and

the probability  $p < 0.05$  indicated significant differences. All statistic analysis was conducted using Microsoft Excel software.

#### V. Calculation of Measurement Uncertainty

Based on Eurachem/CITAC guide<sup>(17)</sup>, the sources of variation during experiment include repeatability/reproducibility, instrumental errors, mass/volume measurement equipments, purity of the chemicals, etc. These components were considered in the calculation of the uncertainty of measurement.

## RESULTS AND DISCUSSION

### I. Concentrations of Fructans after Measurement by AOAC 985.29 for Fructan-containing Foods and Products

In this research, five vegetable sources and three fructan-containing processed foods were tested. The selected materials were analyzed by 985.29, 999.03 and combined methods. Results are shown in Table 1. It is noted that the values of 999.03\* came from the filtrate after the analysis by 985.29. The filtrate was then analyzed by 999.03 to determine the fructan concentration (as presented in Figure 1). It is shown in Table 1 that these eight food products (excluding asparagus) contain certain level of fructan. The highest proportion of fructan in

filtrate was found in tea drink 1 (98.39% of fructan). The lowest proportion was found in leeks, which reaches the value of 28.89%. Based on the Ku *et al.*'s point of view<sup>(14)</sup>, the significant amount of fructans of low degree of polymerization in these eight samples can not completely form sediments using 78% alcohol by 985.29.

Due to the fact that the actual content and the degree of polymerization in tested foods were unknown, some samples with known degree of polymerization were tested. Based on the market demand, fructan with various degrees of polymerization are classified in different categories. For example, the samples with the average degree of polymerization less than 10 are usually applied as low calorie sweeteners; the natural inulin products generally have degree of polymerization in the range of 9 to 12, and the long chain fructans with degree of polymerization larger than 23 can be used for fat substitutes. Thus, three commercial available fructan samples were chosen: Fructafit CLR, Fructafit IQ and Fructafit TEX with the average degrees of polymerization of 7, 12 and 25, respectively. The solutions made from those samples were tested by 985.29, 999.03 and combined methods, and the results were shown in Table 1. The concentrations of three commercial samples detected by 999.03\* are 4.25%, 3.92% and 1.24% respectively. Compared the results from 999.03\* with 999.03, it can be found that the percentage of residual fructan recovered from the filtrate solutions tested after 985.29 method are 101.19%, 85.03% and 25.99%, respectively. These data show that the total

**Table 1.** Results of different dietary fiber analytical methods for fructan-containing foods and commercial fructan products<sup>1</sup>

| Method                             | Fructan (g/100g)         |                      | Total dietary fiber (g/100g) |   |   |
|------------------------------------|--------------------------|----------------------|------------------------------|---|---|
|                                    | 999.03 <sup>4</sup>      | 999.03* <sup>5</sup> | 985.29 <sup>4</sup>          | 985.29 <sup>4</sup> + 999.03 <sup>4</sup> | Combined<br>985.29+999.03* <sup>6</sup> |
| <b>Fructan-containing foods</b>    |                          |                      |                              |   |   |
| Onion                              | 1.65 ± 0.11 <sup>2</sup> | 1.12 ± 0.03          | 2.12 ± 0.06                  | 3.77 ± 0.05                               | 3.24 ± 0.09                             |
| Asparagus                          | 0.08 ± 0.00              | 0.00 ± 0.00          | 1.72 ± 0.11 <sup>a</sup>     | 1.80 ± 0.11 <sup>a</sup>                  | 1.64 ± 0.11 <sup>a</sup>                |
| Leek                               | 0.45 ± 0.00              | 0.13 ± 0.03          | 2.82 ± 0.01                  | 3.27 ± 0.01                               | 2.94 ± 0.04                             |
| Garlic sprout                      | 0.56 ± 0.02              | 0.31 ± 0.03          | 3.40 ± 0.04                  | 3.92 ± 0.03                               | 3.70 ± 0.07                             |
| Burdock                            | 7.53 ± 0.11              | 5.96 ± 0.10          | 4.92 ± 0.07                  | 12.45 ± 0.06                              | 10.88 ± 0.16                            |
| Tea drink 1                        | 1.85 ± 0.03              | 1.82 ± 0.05          | 0.01 ± 0.02                  | 1.87 ± 0.02 <sup>b</sup>                  | 1.83 ± 0.06 <sup>b</sup>                |
| Grain powder                       | 20.46 ± 0.36             | 17.78 ± 0.44         | 9.28 ± 0.10                  | 29.74 ± 0.46                              | 27.06 ± 0.54                            |
| Milk powder                        | 7.71 ± 0.19              | 6.65 ± 0.09          | 4.30 ± 0.08                  | 12.01 ± 0.26                              | 10.95 ± 0.07                            |
| <b>Commercial fructan products</b> |                          |                      |                              |   |   |
| Fructafit CLR solution             | 4.20 ± 0.05              | 4.25 ± 0.04          | 0.02 ± 0.00                  | 4.22 ± 0.05 <sup>c</sup>                  | 4.27 ± 0.04 <sup>c</sup>                |
| Fructafit IQ solution              | 4.61 ± 0.02              | 3.92 ± 0.04          | 0.66 ± 0.02                  | 5.27 ± 0.00                               | 4.58 ± 0.05                             |
| Fructafit TEX solution             | 4.77 ± 0.17              | 1.24 ± 0.00          | 3.67 ± 0.04                  | 8.44 ± 0.18                               | 4.91 ± 0.04                             |

<sup>1</sup>All data were measured in triplicate.

<sup>2</sup>Means ± standard deviations.

<sup>3,a-c</sup>Data with the same letter are not significantly different ( $p > 0.05$ ).

<sup>4</sup>999.03 stands for AOAC 999.03 method, 985.29 stands for AOAC 985.29 method.

<sup>5</sup>999.03\* method stands for the filtrate after the analysis of 985.29 was analyzed by 999.03.

<sup>6</sup>Combined 985.29+999.03\* meaning the combination of 985.29 and 999.03\*.

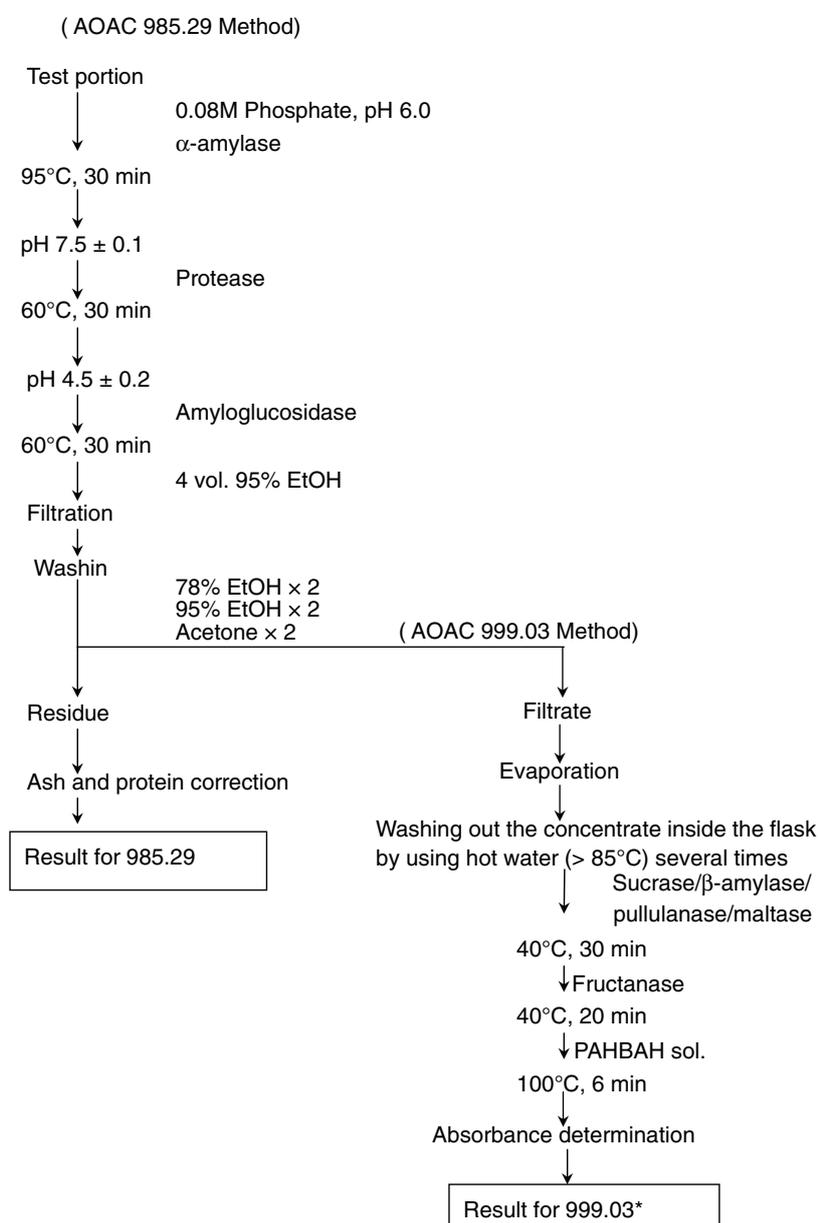


Figure 1. Flow diagram for combined method.

concentration of fructan can not be determined by 985.29 alone, and fructans with lower average degree of polymerization tend to be lost in the filtrate.

## II. Analysis of Fructan-containing Products by Different Analytical Methods for Dietary Fiber

With the presumption of fructan as dietary fiber, the recovery rates of commercial samples from three dietary fiber methods (985.29, the sum of 985.29 plus 999.03 and combined method) are shown in Table 2. The fructan concentrations of Fructafit CLR and Fructafit IQ determined by 985.29 were substantially lower than the actual concentrations; the recoveries were found only 0.41%

and 14.35% respectively. However, there was 75.76% of fructan recovered from Fructafit TEX with higher degree of polymerization by 985.29. The recovery ratio of three fructan-containing solutions by combined method lies in the range of 97.59~101.35%, close to the added concentration of dietary fiber. Assuming the total dietary fiber as the sum by 985.29 and 999.03, the recovery of Fructafit CLR would be 96.81%, Fructafit IQ would have slightly higher recovery of 114.93% and the recovery of Fructafit TEX would be much higher than actual value as 174.08%. Because fructan solution is of higher degree of polymerization, more fructan can be determined in 985.29, thus, causing over-estimation of fructans with higher degree of polymerization.

**Table 2.** Recoveries of different dietary fiber analytical methods for commercial fructan products<sup>1</sup>

| Samples                | Average DP | Amount added<br>(g/100g) | Recovery (%)        |   |   |
|------------------------|------------|--------------------------|---------------------|---|---|
|                        |            |                          | 985.29 <sup>2</sup> | 985.29 <sup>2</sup> + 999.03 <sup>2</sup> | Combined<br>985.29 + 999.03* <sup>3,4</sup> |
| Fructafit CLR solution | 7          | 4.36                     | 0.41                | 96.81                                     | 97.59                                       |
| Fructafit IQ solution  | 12         | 4.56                     | 14.35               | 114.93                                    | 99.76                                       |
| Fructafit TEX solution | 25         | 4.86                     | 75.76               | 174.08                                    | 101.35                                      |

<sup>1</sup>All data were measured in triplicate.

<sup>2</sup>999.03 stands for AOAC 999.03 method, 985.29 stands for AOAC 985.29 method.

<sup>3</sup>999.03\* method stands for the filtrate after the analysis of 985.29 was analyzed by 999.03.

<sup>4</sup>Combined 985.29+999.03\* meaning the combination of 985.29 and 999.03\*.

### III. Calculation of Dietary Fiber Concentration and Calorie in Fructan-containing Foods

There are currently two ways in determining total dietary fiber content: one only considers the value detected by 985.29, and the other includes fructan as part of total dietary fiber which is the sum of values from 985.29 plus 999.03. However, it is found that there is a redundant measurement problem for the later method. Based on the results from Table 1, the values of total dietary fiber content of fructan-containing foods measured by 985.29 are significantly lower than the sum of 985.29 plus 999.03, or combined method (except asparagus), and the sum of 985.29 plus 999.03 are significantly higher than combined method (except tea drink 1 and asparagus). It is plausible that asparagus contains low fructan (only 0.08% by 999.03), thus no significant differences among 985.29, the sum of 985.29 plus 999.03 and combined method. Also, it is plausible that values of dietary fiber from 985.29 are very low in tea drinks 1 (0.01% in Table 1). Although the difference is found ( $p < 0.05$ ) between 985.29 and the sum of 985.29 plus 999.03, there is no significant difference between the sum of 985.29 plus 999.03 and the combined method.

The results for fructan solutions demonstrate that Fructafit CLR is very low in 985.29, making there is no statistically differences between values from the sum of 985.29 plus 999.03 and the combined method. In short, despite certain dietary fiber foods, the combined method should be employed in most fructan containing products when the total accurate dietary fiber content is concerned.

Recently, the Department of Health (DOH) had made an announcement enforcing the nutrition labeling of packaged foods, which should be closely related to the definition and calorie calculation of total dietary fiber. However, the calculation of calorie has not been reached a unified agreement worldwide. In Taiwan, Japan and Korea, the calorie of dietary fiber is currently defined by 985.29 as 0 kcal/g, and other carbohydrates as 4 kcal/g. However, based on the review from Flamm *et al.*, calorie of fructan should lie in the range of 1.5~2.0 kcal/g<sup>(16)</sup>. Thus, assuming total dietary fiber based on 985.29 and those fructan does not form sediments in 985.29 as carbohydrates, the

calories counts would be higher than actual. On the other hand, if considering both values from 985.29 plus 999.03 as dietary fiber and calorie as 0 kcal/g, results might be lower than expected due to redundant quantification. Thus, based on the results shown above, it is suggested to calculate the calorie of fructan-containing foods based on combined method: 985.29 and 999.03\*. Because 999.03\* measured the filtrate of 985.29, it does not have the problem of redundant quantification. Therefore, when calculating calories, it is suggested to calculate total dietary fiber as the sum of values from 985.29 as 0 kcal/g with addition of 999.03\* as 1.5~2.0 kcal/g.

### IV. Validation of the Method

#### (I) Accuracy

The importance of accuracy of combined method was highlighted. CRM 381 rye flour was tested to evaluate the accuracy of 985.29 method. The average concentration of CRM 381 rye flour is  $8.18 \pm 0.12\%$  ( $n = 7$ ), which lies in certified value of 8.0~8.4%, showing high accuracy of the method. We also evaluate the accuracy of 999.03 using Fructan control powder (containing 28.8% of fructan) by Megazyme. The result also shows high accuracy with the recovery of  $99.82 \pm 0.64\%$  ( $n = 7$ ). The accuracy of combined method is shown in Result and Discussion II and the recovery was in the range of 98.28~101.07%. Moreover, tea drink 2 (liquid) and asparagus (solid) were selected and spiked to determine the recovery of products. Result are shown in Table 3, that the recoveries of the product are  $97.02 \pm 0.63\%$  and  $97.96 \pm 0.46\%$  respectively. High accuracy of the method regarding the degree of polymerization and the matrix of the products is also shown.

#### (II) Precision

Three commercial samples were tested for the precision of combined method: Fructafit CLR, Fructafit IQ and Fructafit TEX. Samples were made with known concentration for each method and tested in triplicate. The concentration for Fructafit CLR was 4.36%; Fructafit IQ

**Table 3.** Evaluation of accuracy for combined method<sup>1</sup>

| Samples     | Blank (mg/g) | Amount added (mg/g) | Amount found (mg/g) | Recovery (%)              | CV (%) |
|-------------|--------------|---------------------|---------------------|---------------------------|--------|
| Tea drink 2 | 0.00         | 20.33               | 19.67               | 97.02 ± 0.63 <sup>2</sup> | 0.65   |
| Asparagus   | 16.4         | 91.86               | 105.94              | 97.96 ± 0.64              | 0.63   |

<sup>1</sup>. All data were measured in triplicate.

<sup>2</sup>. Means ± standard deviations.

**Table 4.** Evaluation of precision for combined method<sup>1</sup>

| Samples                       | Total dietary fiber (g/100g) | CV (%) |
|-------------------------------|------------------------------|--------|
| 4.36 % Fructafit CLR solution | 4.27 ± 0.04 <sup>2</sup>     | 0.86   |
| 4.56 % Fructafit IQ solution  | 4.58 ± 0.07                  | 1.17   |
| 4.86 % Fructafit TEX solution | 4.91 ± 0.04                  | 0.76   |

<sup>1</sup>. All data were measured in triplicate.

<sup>2</sup>. Means ± standard deviations.

was 4.56% and Fructafit TEX was 4.86%. The results are shown in Table 4. The CV for the testing solutions was 0.86%, 1.17% and 0.76% respectively which demonstrates high precision of the combined method.

### (III) Measurement Uncertainty

The measurement of uncertainty was based on the calculation according to Eurachem/CITAC Guide<sup>(17)</sup>. Take burdock as an example, the measurement of uncertainties for 985.29, 999.03 and the combined method were 4.88 ± 0.42%, 7.49 ± 0.24% and 10.87 ± 0.71%, respectively. The uncertainty for all three methods lies within acceptable range, and the uncertainty for combined method was slightly higher than that of 985.29 or 999.03 because of the additional steps in combined method.

## CONCLUSIONS

With the intense development and research in food ingredients and components, the definition scope and analytical methods for technical words should also be updated periodically. Take dietary fiber for example, other than the testing of AOAC 985.29, only three types of dietary fibers own official analyzing methods including fructans, resistant maltodextrin and polydextrose. Based on the results above, the combined method not only can determine the concentration of fructans, but also is able to provide a more accurate and comprehensive calculation for total dietary fiber content and calorie.

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