Rapid Determination of Dietary Components in Nutritional Drinks

Introduction

Over the past 20 years, public interest in nutraceuticals has rapidly grown. Many are turning to nutritional drinks as meal replacements and dietary supplements. A variety of factors influence purchasing and consumption of these beverages. One such area of focus includes the added nutritional value these drinks offer to a person's diet in the form of protein, amino acids, and/or fat content. Furthermore, it is crucial for the manufacturer to analyze and control these major components in order to optimize production, control costs, and produce high-quality products.

Traditional methods often rely on tedious and time-consuming gravimetric wet chemistry techniques.[1] Alternatively, CEM offers a suite of instrumentation to address these needs in a more efficient, reliable, and environmentally friendly manner. As an example, reference methods for crude fat analyses include Soxhlet, Babcock, and Mojonnier extraction. These techniques rely on the addition of solvent to dissolve and separate the desired fat from other components in the sample matrix and are often accompanied by acid/base hydrolysis. The ORACLE™ represents an attractive alternative to these methods, measuring fat without calibration or method development for any sample type. The ORACLE can be paired with the SMART 6™ for direct, accurate solids and fat determination in minutes.

Total protein determination also relies on harsh Kjeldahl sulfuric acid digestion, followed by titration or combustion methods to determine total nitrogen content. The Sprint® uses dye-binding technology, wherein CEM's iTag® dye molecule selectively binds to basic amino acid side chains in protein. Not only does this offer a targeted approach, but also allows testing to be completed in under five minutes, with reagents that do not require chemical waste disposal.

In addition to total protein measurements, the quantity and identity of the amino acid residues that make up these proteins are also of importance. To analyze these moieties, protein amide bonds must be cleaved in order to liberate the individual amino acids. Samples are typically treated under acidic or alkaline conditions at 110 °C for 18 to 24 hours and the resulting hydrolysate is analyzed via LC-UV or LC-MS techniques. Characteristic of conventional heating mechanisms, long reaction times and unwanted side reactions plague traditional oven methods. CEM's Discover® Prep microwave reactor offers an excellent path forward for the optimization of amino acid hydrolysis (AAH) reactions in a more rapid and efficient manner.[2,3]

Herein, various nutritional drinks were analyzed for total fat, solids, and protein content, as well as prepared for LC-UV amino acid analysis using CEM instrumentation. Overall, these results compared well to their respective traditional wet chemistry techniques but were achieved in a more rapid, precise, and safe manner.

Materials and Methods

Experimental Details

Chemicals were purchased from commercial suppliers and used without further purification. All solutions were freshly prepared prior to use. Various nutritional beverages were purchased from a local grocery store and stored in a dry and cool location, prior to analysis. All samples were analyzed, as is and stored at 4 °C once opened.

Analysis

Analytical results obtained from the SMART 6, ORACLE, and Sprint were compared to traditional wet chemistry methods obtained from the corresponding AOAC reference methods for solids (990.20), fat (989.05), and protein (981.10), respectively.

The effectiveness of the Discover Prep was compared to a traditional air oven. Briefly, 200 mg portions of nutritional drinks were pipetted into clean and dry 10-mL glass vials, followed by 2 mL of 6 N HCl with 1% phenol (w/v). Vials were then purged with N_2, quickly sealed, and then placed into a 110 °C air oven for 24 hours. Tryptophan was analyzed, following a base hydrolysis protocol, wherein 4 N NaOH was used in lieu of HCl. Discover Prep microwave-assisted AAH reactions were prepared as follows: a 200 mg sample portion was added to a 35-mL Pyrex vial equipped with an egg-shaped stir bar. A volume of 5 mL of 6 N HCl with 1% phenol (w/v) or 4 N NaOH was aliquoted into each vial for acid or base hydrolysis, respectively. Vials were purged with a stream of N_2 for five minutes, quickly sealed with a Teflon® lined silicon cap, and placed in the autosampler rack for automated placement in the Discover Prep cavity. The Discover Prep AAH method parameters are shown in **Table 1** (page 2). Upon the completion of the oven and microwave reactions, the cooled samples were neutralized and filtered in preparation for pre-column derivatization. All amino acid hydrolysates were derivatized using Waters AccQ-Tag™ Ultra Derivatization kit and analyzed, following injection onto a Waters AccQ-Tag Ultra C18 column (1.7 µm, 2.1 x 100 mm) attached to a Waters Acquity H-Class UPLC with PDA detection at 260 nm.[4]

Table 1. Discover Prep Amino Acid Hydrolysis Parameters

Acid Hydrolysis	Base Hydrolysis
Vial Type: Pyrex	Vial Type: Pyrex with Liner
Control Type: Dynamic	Control Type: Dynamic
Temperature: 160 °C	Temperature: 190 °C
Time: 30-min	Time: 30-min
Pressure: 300 PSI	Pressure: 250 PSI
Power: 300 W	Power: 300 W
Stirring: High	Stirring: High

Results and Discussion

Table 2 and **Table 3** depict total solids and fat results obtained from the SMART 6 and ORACLE, respectively. In a variety of nutritional drinks, results compared very well to traditional air oven drying for solids and Mojonnier ether extraction for fat determination. In all cases, results from CEM's instrumentation match traditional methods, within a range of ±0.10%, regardless of the sample composition. Further, results were comparable in terms of precision, with all percent relative standard deviations (%RSDs) remaining under 1%.

The Sprint also excelled when analyzing adult and pediatric nutritional beverage flavors, as seen in **Table 4**. In this case, Sprint results displayed lower %RSDs than the traditional Kjeldahl method. Further, when challenged with samples formulated with a variety of protein sources, including pea protein, milk protein isolate, and whey protein, the Sprint proved successful.

Table 2. Comparison of Results Obtained Using the SMART 6 vs. Air Oven Drying Method for Percent Solids in Various Nutritional Drinks

	%Solids				
Sample	SMART 6	%RSD	Oven	%RSD	Diff.
Adult Nutritional Drink, Vanilla	19.96	0.15	19.91	0.00	0.05
Adult Nutritional Drink, Chocolate	20.33	0.37	20.42	0.48	-0.09
Pediatric Nutritional Drink, Vanilla	20.24	0.33	20.31	0.31	-0.07
Pediatric Nutritional Drink, Fruit and Vegetable	21.72	0.03	21.67	0.03	0.05
Diabetic Nutritional Drink, Strawberry	14.57	0.00	14.51	0.01	0.06
Diabetic Nutritional Drink, Chocolate	14.56	0.03	14.57	0.00	-0.01
Diabetic Nutritional Drink, Vanilla	14.42	0.15	14.32	0.10	0.09

Table 3. Comparison of Results Obtained using the ORACLE vs. Mojonnier Fat Extraction Method for Percent Fat in Various Nutritional Drinks

	%Fat				
Sample	ORACLE	%RSD	Mojo.	%RSD	Diff.
Adult Nutritional Drink, Vanilla	13.38	0.41	13.46	0.37	-0.08
Adult Nutritional Drink, Chocolate	2.30	0.25	2.40	0.80	-0.10
Pediatric Nutritional Drink, Vanilla	3.56	0.90	3.54	0.00	0.02
Pediatric Nutritional Drink, Fruit and Vegetable	3.91	0.39	3.91	0.72	0.00
Diabetic Nutritional Drink, Strawberry	2.81	0.62	2.81	0.08	0.00
Diabetic Nutritional Drink, Chocolate	2.69	0.43	2.63	0.27	0.06
Diabetic Nutritional Drink, Vanilla	2.83	0.41	2.83	1.00	0.00

Table 4. Comparison of Results Obtained Using the Sprint vs. Kjeldahl Method for Percent Protein in Various Nutritional Drinks

		%Protein				
Sample	Protein Source	Sprint	%RSD	Kjeldahl	%RSD	Diff.
Adult Nutritional Drink, Vanilla	Milk Protein Isolate	11.36	0.00	11.35	0.02	0.01
Adult Nutritional Drink, Chocolate	Milk Protein Isolate	8.37	0.00	8.35	0.02	0.02
Adult Nutritional Drink, Vanilla	Pea Protein	4.67	0.12	4.66	0.01	0.01
Pediatric Nutritional Drink, Unflavored	Pea Protein	4.59	0.25	4.63	0.46	-0.04
Adult Nutritional Drink, Chocolate	Soy and Milk Protein Isolate	5.57	0.10	5.58	0.63	0.01
Adult Nutritional Drink, Vanilla	Soy and Milk Protein Isolate	5.56	0.54	5.58	0.00	0.02
Adult Nutritional Drink, Chocolate	Pea and Rice Protein Isolate	4.25	0.85	4.21	1.34	0.04
Adult Nutritional Drink, Vanilla	Pea and Rice Protein Isolate	4.12	0.74	4.11	0.86	0.01

For amino acid hydrolysis, a chocolate and vanilla adult nutritional drink was selected for analysis. Samples were subjected to acidic and alkaline hydrolysis in preparation for the LC-UV analysis of 18 amino acids. Results achieved using the Discover Prep microwave reactor were comparable in terms of accuracy and precision to their traditional oven hydrolysis counterparts, resulting in acceptable recoveries ranging between 80 and 120% (**Figure 1** and **Figure 2** on page 3).

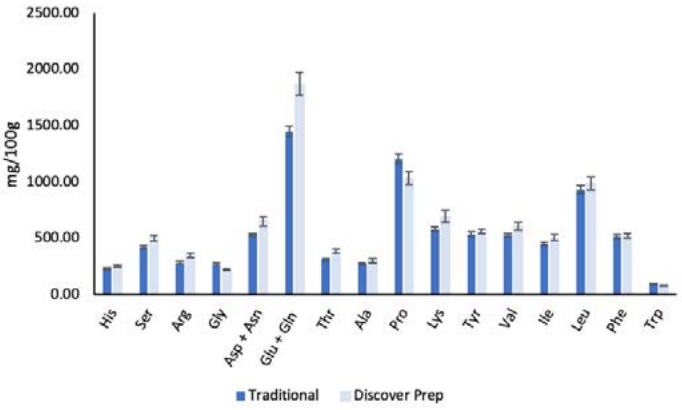

Figure 1. Amino Acid Hydrolysis Recoveries for 18 Amino Acids in a Chocolate Nutritional Drink

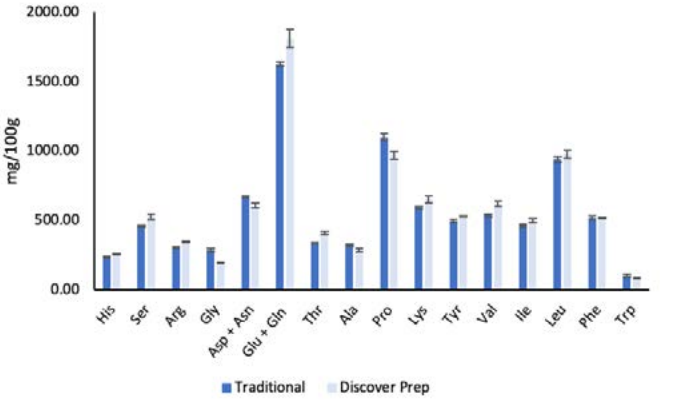

Figure 2. Amino Acid Hydrolysis Recoveries for 18 Amino Acids in a Vanilla Nutritional Drink

Conclusion

Consumer interest in the nutritional value of food significantly influences the production, regulation, and distribution of products around the world. This, in conjunction with stringent regulatory requirements, highlights an ever-growing need for research into efficient, reliable, and robust methods for compositional analyses. Conventional methods for such analyses are typically time-consuming, often taking several hours and requiring harsh chemicals. The SMART 6, ORACLE, and Sprint successfully analyzed total solids, fat, and protein content in nutritional drinks in a more efficient manner than traditional wet chemistry. The Discover Prep was used to successfully prepare various nutritional beverages for LC-UV analysis of 18 amino acids. In regards to accuracy and precision, the Discover Prep was comparable to traditional oven hydrolysis methods. Additional key benefits of CEM's suite of instrumentation include easy-to-use software and safer laboratory protocols, while maintaining accuracy and precision.

References

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[3] Dudley, G. B.; Richert, R.; Stiegman, A. E. On the existence of and mechanism for microwave-specific reaction rate enhancement. *Chem. Sci.* **2015**, *6*, 2144-2152.

[4] Hong, P.; Johnson, D.; Trinite, D. A.; Warren, B.; Zhang, N. *Hydrolysis and Analysis of Amino Acids from Purified Peptides/ Proteins, Foods, and Feeds*; Waters Corporation, 2019.

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