

# Evaluation of Carbohydrate Quantification Modalities for Certifying Ketogenic Products

Karen Pendergrass <sup>1</sup>  Kimberly Eyer <sup>2</sup> 

Pendergrass, K., 2023. **Evaluation of Carbohydrate Quantification Modalities for Certifying Ketogenic Products.** *Ketogenic Diet Research*. The Paleo Foundation.

<sup>1</sup>Department of Standards, Paleo Foundation, Encinitas, CA

## Correspondence

Karen E. E. Pendergrass  
Department of Standards, Paleo Foundation, Encinitas, CA

## Contact

<sup>1</sup>Email: karen@paleofoundation.com

<sup>1</sup>Twitter: @5WordsorlessKP

## KEYWORDS

Ketogenic Diet  
Carbohydrate Quantification  
Net Carbohydrates  
High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)  
Glycemic Index (GI)  
Product Certification

## KETOGENIC DIET RESEARCH

# Evaluation of Carbohydrate Quantification Modalities for Certifying Ketogenic Products

Karen E. E. Pendergrass<sup>1</sup>Kimberly L. Eyer<sup>2</sup><sup>1</sup> Department of Standards, Paleo Foundation, Encinitas, CA<sup>2</sup> Department of Auditing, Paleo Foundation, Orlando, FL**Correspondence**Karen E. E. Pendergrass  
Department of Standards, Paleo Foundation, Encinitas, CA**Contact**<sup>1</sup>Email: [karen@paleofoundation.com](mailto:karen@paleofoundation.com)<sup>1</sup>Twitter: [@5WordsorlessKP](https://twitter.com/5WordsorlessKP)

## Abstract

This review aims to determine the most appropriate method for quantifying net carbohydrates to certify ketogenic products. Two standard methods of carbohydrate analysis, the Glycemic Index (GI) and High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD), were evaluated for their suitability in this context. The review reveals that while the GI provides valuable insights into how food affects blood sugar levels, it does not offer the detailed carbohydrate quantification necessary for ketogenic product certification. Conversely, HPAEC-PAD, despite its complexity and cost, provides a precise and comprehensive analysis of individual carbohydrates, enabling accurate calculation of net carbohydrates.

**KEYWORDS**

Ketogenic Diet, Carbohydrate Quantification, Net Carbohydrates, High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection, Glycemic Index, Product Certification.

## 1 | INTRODUCTION

The ketogenic diet, characterized by low carbohydrate, moderate protein, and high fat intake, has gained popularity as a dietary approach for weight management, metabolic health, and various medical conditions. Central to this diet is the concept of ketosis, a metabolic state where the body primarily burns fats for energy due to the restricted carbohydrate intake. To achieve and maintain ketosis, individuals following a ketogenic diet must carefully monitor their net carbohydrate intake, typically limiting it to 20-50 grams daily. Net carbohydrates are calculated by subtracting the grams of fiber and sugar alcohols from the total carbohydrates, as these components are not digested and absorbed like other carbohydrates. Accurate quantification of net carbohydrates in food products is therefore crucial for individuals following a ketogenic diet.

The increasing popularity of the ketogenic diet has led to a surge in demand for ketogenic food products. As a result, there is a growing need for reliable methods to certify these products as ketogenic-friendly. Two standard methods used for carbohydrate analysis are the Glycemic Index (GI) and High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The GI measures how quickly and how much a food raises blood glucose levels, while HPAEC-PAD is a sophisticated analytical technique used to quantify individual carbohydrates in a sample. However, each method has limitations and strengths, and their suitability for ketogenic product certification requires careful consideration.

This review aims to evaluate the suitability of the GI and HPAEC-PAD for net carbohydrate quantification in

the certification of ketogenic products. It will explore the strengths and limitations of each method, assess their suitability for ketogenic product certification, and provide recommendations for the most appropriate practice for this purpose.

## 2 | METHODOLOGY

### GLYCEMIC INDEX

The Glycemic Index (GI) is a measure that ranks foods containing carbohydrates on a scale from 0 to 100 based on how quickly and how much they raise blood glucose levels after being consumed. Here is the typical process for measuring the GI of a food:

**1. Selection of Subjects:** Healthy individuals or people with controlled diabetes usually participate in GI testing.

**2. Fasting:** Participants are usually required to fast overnight, typically for at least 10-12 hours, to ensure their blood sugar levels are at baseline.

**3. Baseline Blood Sample:** A baseline blood sample is taken before the test food is consumed.

**4. Consumption of Test Food:** The test food containing a fixed amount of available carbohydrates (usually 50 grams) is consumed. The food must be consumed within a specified period, usually 5-15 minutes.

**5. Blood Sampling:** Multiple blood samples are taken regularly after the test food is consumed, typically at 15, 30, 45, 60, 90, and 120 minutes. These samples are used to measure the blood glucose response to the test food.

**6. Reference Food:** For comparison, the same process is repeated on a different day using a reference food (glucose or white bread) containing the same amount

of available carbohydrates as the test food. The reference food is assigned a GI value of 100.

**7. Calculation of GI:** The area under the blood glucose response curve (AUC) for the test food is compared to the reference food. The GI of the test food is then calculated using the following formula:

$$GI = \left( \frac{\text{AUC of test food}}{\text{AUC of reference food}} \right) \times 100$$

The resulting value is the GI of the test food. Foods are usually classified as low ( $GI \leq 55$ ), medium ( $56 \leq GI \leq 69$ ), or high ( $GI \geq 70$ ) glycemic index foods.

It is important to note that the GI of a food can be significantly influenced by several factors, including the type and amount of carbohydrates, the cooking process, the presence of other nutrients (e.g., fat, protein, fiber), and individual differences in metabolism.

It has been recognized since the 1970s that carbohydrate-containing foods elicit widely different postprandial blood glucose responses [1, 2].

While the GI classification system has been endorsed as a tool to help guide food choices by some individuals and organizations, others have shared reluctance to universally recommend the system for use in formulating dietary guidance due to several issues regarding uncertainties in reproducibility and variability among individual responses to foods [3, 4, 5].

### HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (HPAEC-PAD)

High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) is a technique used for the separation and quantification of carbohydrates (monosaccharides, disaccharides, and some oligosaccharides) in a sample. Here's how the process typically works:

**1. Sample Preparation:** The sample to be analyzed is prepared by extracting the carbohydrates from the food or beverage. This step may involve homogenizing the sample, followed by filtration or centrifugation to remove solid particles. The sample may also be diluted to fall within the appropriate concentration range for the analysis.

**2. Chromatographic Separation:** The prepared sample is injected into a chromatographic column packed with an anion-exchange resin. This column is placed in a high-performance liquid chromatography (HPLC) system. An eluent (mobile phase) is pumped through the column at high pressure to facilitate the separation of the carbohydrates. The eluent is typically a mixture of water and a strong electrolyte, such as sodium hydroxide.

**3. Anion-Exchange Process:** The carbohydrates in the sample are separated based on their interaction with the anion-exchange resin in the column. The resin contains positively charged groups that interact with the negatively charged hydroxyl groups of the carbohydrates. Different carbohydrates have different numbers and positions of hydroxyl groups, leading to varying affinities for the resin and, therefore, different movement rates through the column.

**4. Detection:** As the carbohydrates are eluted from the column, they are detected using pulsed amperometric detection (PAD). PAD involves applying a sequence of voltage pulses to an electrode and measuring the resulting current. Carbohydrates are electroactive and can be oxidized at the electrode surface, leading to a

current that is proportional to their concentration. The PAD detector generates a chromatogram, which is a plot of the current (or response) versus time.

**5. Quantification:** The area under the peaks in the chromatogram is proportional to the amount of each carbohydrate in the sample. The concentration of each carbohydrate in the sample can be quantified by comparing the area of each peak to a calibration curve generated using known concentrations of standards.

HPAEC-PAD is a highly sensitive, selective, and accurate technique for carbohydrate analysis. It can separate and quantify individual monosaccharides, disaccharides, and some oligosaccharides, providing a comprehensive carbohydrate profile of a sample. This makes it particularly useful for determining the net carbohydrate content of a food or beverage.

### 3 | COMPARISON OF METHODS: UTILITIES AND LIMITATIONS OF GI AND HPAEC-PAD

#### GLYCEMIC INDEX (GI):

**Characteristics:** The GI is a ranking system that categorizes carbohydrate-containing foods based on their potential to elevate blood glucose levels. Foods are rated on a scale of 0-100, with higher values indicating a more rapid rise in blood glucose.

**Utility:** The GI is a valuable tool for managing blood sugar levels, particularly for individuals with diabetes or pre-diabetes. It allows these individuals to make dietary choices to help control their blood glucose levels.

**Limitations:** As mentioned earlier, the GI has several limitations. First, it does not account for the quantity of carbohydrates consumed, merely their type. A food may have a low GI, but if consumed in large amounts, it can still significantly affect blood glucose levels. Second, the GI does not consider other food components, such as fiber, fat, and protein, which can alter the food's overall effect on blood sugar. Third, GI does not account for individual variability in glycemic response, which can be influenced by factors such as age, physical activity, and gut microbiota. Finally, the GI does not account for the amount of carbohydrates consumed, which is a limitation when determining the suitability of a food for specific dietary needs. For example, a food could have a low GI but still contain a high number of carbohydrates, which could affect an individual's ability to maintain ketosis on a ketogenic diet.

However, one of the greatest limitations to the utility of the Glycemic index model within the context of ketogenic product certification lies in the the limitations of reproducibility, discussed further in the next section.

#### REPRODUCIBILITY OF GLYCEMIC INDEX (GI):

**Individual Variability:** There is significant inter-individual variability in glycemic responses. Different people can have different glycemic responses to the same food due to factors like genetics, insulin sensitivity, and gut microbiome composition. This means that a food's GI might not accurately predict its impact on blood sugar for every individual [6].

**Food Preparation and Processing:** The way a food is prepared and processed can alter its glycemic response. For example, pasta that is cooked al dente has a lower GI than pasta that is cooked until soft. The same food can have different GI values depending on its preparation [7].

**Mixed Meals:** The GI is determined by testing foods in isolation. However, the glycemic response can be different when a food is consumed as part of a mixed meal. For example, adding fat or protein to a carbohydrate-rich food can lower its glycemic response [8].

**Carbohydrate Quality:** The GI does not take into account the type of carbohydrate present in the food. For example, fructose has a lower GI than glucose, but a high intake of fructose can lead to other metabolic issues [9].

**Day-to-Day Variability:** There is also day-to-day variability in glycemic responses. The same person can have a different glycemic response to the same food on different days [10].

#### UNSUITABLE APPLICATIONS OF THE GI

These issues limit the reproducibility and generalizability of the GI, making it less reliable for individual dietary recommendations or for determining the suitability of a food product for a specific diet, like the ketogenic diet.

Using the GI to evaluate food products for their ketogenic suitability or for precisely quantifying carbohydrates is not ideal. The ketogenic diet requires strict monitoring of carbohydrate intake, for which the GI is insufficient.

#### HIGH-PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY WITH PULSED AMPEROMETRIC DETECTION (HPAEC-PAD):

**Characteristics:** HPAEC-PAD is an analytical technique that separates and quantifies individual carbohydrates in a sample. This method can identify

and measure monosaccharides, disaccharides, and some oligosaccharides with high sensitivity and accuracy.

**Utility:** HPAEC-PAD is an excellent tool in research and industry for detailed carbohydrate analysis. It's used in the food and beverage industries for quality control, product development, and nutritional labeling. It's also crucial in academic research for carbohydrate-related studies.

**Limitations:** HPAEC-PAD requires specialized equipment and trained personnel. It also involves a longer and more complex process compared to other simpler carbohydrate tests. Additionally, the cost associated with this method can be significantly high, making it less accessible for routine or large-scale use.

## REPRODUCIBILITY OF HPAEC-PAD:

High-performance anion-exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) is a highly sensitive and selective method for the analysis of carbohydrates. However, like any analytical technique, it has its limitations and potential reproducibility issues.

**Sample Preparation:** The sample preparation process can affect the reproducibility of the results. For example, incomplete or inconsistent sample extraction can lead to variability in the results [11].

**Matrix Effects:** The presence of other compounds in the sample matrix can interfere with the separation and detection of carbohydrates, affecting the reproducibility and accuracy of the results [12].

**Instrumental Variability:** Variability in the performance of the chromatography system and the detector can affect the reproducibility of the results. For example, changes in the flow rate, column temperature, or detector response can lead to variability in the results.

**Column Aging:** Over time, the chromatography column can degrade leading to changes in the separation efficiency and, consequently, the reproducibility of the results [13].

While HPAEC-PAD is a powerful technique for the analysis of carbohydrates, it is essential to have rigorous quality control procedures in place in the laboratories to minimize these potential sources of variability and ensure the reproducibility of the results.

## UNSUITABLE APPLICATIONS:

Given its complexity and cost, HPAEC-PAD may not be suitable for routine food testing in small businesses, home use, or where a quick, albeit less accurate, estimate of carbohydrates is required.

## 4 | AN EXAMINATION OF THE COMPARATIVE UTILITY OF HPAEC-PAD AND GI IN ANALYZING CARBOHYDRATES FOR KETOGENIC SUITABILITY

The realm of carbohydrate analysis boasts a variety of methods, each with unique applicability and limitations. Two such methods discussed in this review – the glycemic index (GI) and High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) – are employed frequently in different contexts, yet their utilities vary.

The GI was developed as a tool to quantify the impact of a food product on blood glucose levels. Foods that register high GI values undergo rapid digestion and absorption, triggering swift surges in blood sugar. Conversely, foods with low GI values demonstrate a slower digestion-absorption pattern,

culminating in a more gradual change in blood glucose levels.

Notwithstanding, the GI's aptitude in the sphere of carbohydrate quantification exhibits notable drawbacks. The GI metric does not measure the exact amount of carbohydrates in a product; rather, it reflects the estimated impact of food on blood glucose levels. This measurement is swayed by numerous variables, including the type and structure of carbohydrates, the presence of other macro- and micro-nutrients, the preparation process, and even the individual's metabolic response. Hence, using the GI to ascertain net carbohydrate content is susceptible to inaccuracies.

In contrast, HPAEC-PAD emerges as a sophisticated analytical technique explicitly designed to quantify carbohydrates. The method facilitates the separation and identification of mono-, di-, and certain oligosaccharides, thereby furnishing a comprehensive carbohydrate profile of a product. The technique's high sensitivity, selectivity, and precision render it an excellent choice for calculating net carbohydrates.

"Net carbohydrates" typically refers to the total carbohydrate content minus fiber and sugar alcohols, elements known for their marginal impact on blood glucose levels. As such, HPAEC-PAD's ability to effectively differentiate and quantify these components contributes to its superiority for net carbohydrate calculation.

Despite its strengths, HPAEC-PAD entails a complex methodology and significant financial investment, necessitating specialized equipment and trained personnel. This reduces its feasibility for routine application compared to simpler carbohydrate tests.

Although helpful in gauging population-wide blood glucose impacts, the GI fails as an accurate tool for determining a product's suitability for a ketogenic diet. The GI does not account for the total carbohydrate

content, the influence of food pairing, inter-individual variability, all carbohydrate types, or protein content – all of which have potential implications for maintaining a state of ketosis.

Therefore, the net carbohydrate content (total carbohydrates minus fiber and sugar alcohols) emerges as a more dependable metric for assessing a product's ketogenic compatibility. This parameter can be more accurately measured through techniques such as HPAEC-PAD, offering a clearer impression of the potential influence of a food product on ketosis.

## 5 | DISCUSSION

Based on the differences between the two modalities, the selection between the GI and HPAEC-PAD should be dictated by the context and specific requirements of carbohydrate analysis. While the GI is more suited for guiding dietary choices for blood sugar control in an individual, HPAEC-PAD provides a superior tool for detailed, accurate carbohydrate analysis in research, industrial settings, and commercial settings, such as in evaluating a food product's ketogenic compatibility.

Therefore, for evaluating food products' ketogenic compatibility, the net carbohydrate content measured by HPAEC-PAD emerges as a more reliable metric. This is a crucial implication for food industries, dietitians, retailers, and consumers adhering to a ketogenic diet.

For a company certifying Keto products, it is critical to have the most accurate and detailed quantification of net carbohydrates. Net carbohydrates are calculated by subtracting fiber and sugar alcohols from the total carbohydrates, which are not typically digested and absorbed like other carbohydrates



HPAEC-PAD (High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection) would be the more appropriate method for net carbohydrate quantification in this context. This analytical technique can accurately quantify individual carbohydrates (including monosaccharides, disaccharides, and some oligosaccharides) in a product, allowing for a precise calculation of net carbohydrates.

While the Glycemic Index (GI) provides valuable information about how a food might affect blood sugar levels, it does not provide detailed information about the specific types and quantities of carbohydrates in a product. It is, therefore, unsuitable for the precise calculation of net carbohydrates required for ketogenic product certification.

Thus, HPAEC-PAD would be the recommended modality for certifying Keto products for net carbohydrate quantification.

## 5 | REFERENCES

1. Crapo PA, Reaven G, Olefsky J. Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes*. 1976 Sep;25(9):741-7. PMID: 955301. <https://pubmed.ncbi.nlm.nih.gov/955301/>
2. Phyllis A Crapo, Gerald Reaven, Jerrold Olefsky; Postprandial Plasma-glucose and -insulin Responses to Different Complex Carbohydrates. *Diabetes* 1 December 1977; 26 (12): 1178–1183. [doi: 10.2337/diab.26.12.1178](https://doi.org/10.2337/diab.26.12.1178)
3. Pi-Sunyer FX. Glycemic index and disease. *Am J Clin Nutr*. 2002 Jul;76(1):290S-8S. [doi: 10.1093/ajcn/76.1.264S](https://doi.org/10.1093/ajcn/76.1.264S)
4. Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell JQ, Wheeler M; American Diabetes Association. Nutrition principles and recommendations in diabetes. *Diabetes Care*. 2004 Jan;27 Suppl 1:S36-46. [doi: 10.2337/diacare.27.2007.s36](https://doi.org/10.2337/diacare.27.2007.s36).
5. American Heart Association Nutrition Committee; Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, Franklin B, Kris-Etherton P, Harris WS, Howard B, Karanja N, Lefevre M, Rudel L, Sacks F, Van Horn L, Winston M, Wylie-Rosett J. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation*. 2006 Jul 4;114(1):82-96. [doi: 10.1161/circulationaha.106.176158](https://doi.org/10.1161/circulationaha.106.176158)
6. Bouché C, Rizkalla SW, Luo J, Vidal H, Veronese A, Pacher N, Fouquet C, Lang V, Slama G *Diabetes care*, 2002, 25(5), 822-828 | added to CENTRAL: 30 April 2003 | 2003 Issue 2 [doi: 10.2337/diacare.25.5.822](https://doi.org/10.2337/diacare.25.5.822)
7. Wolever TM, Jenkins DJ, Jenkins AL, Josse RG. The glycemic index: methodology and clinical implications. *Am J Clin Nutr*. 1991 Nov;54(5):846-54. [doi: 10.1093/ajcn/54.5.846](https://doi.org/10.1093/ajcn/54.5.846).
8. Wolever TM, Nuttall FQ, Lee R, Wong GS, Josse RG, Csimas A, Jenkins DJ. Prediction of the relative blood glucose response of mixed meals using the white bread glycemic index. *Diabetes Care*. 1985 Sep-Oct;8(5):418-28. [doi: 10.2337/diacare.8.5.418](https://doi.org/10.2337/diacare.8.5.418).
9. Sievenpiper JL, Carleton AJ, Chatha S, Jiang HY, de Souza RJ, Beyene J, Kendall CW, Jenkins DJ. Heterogeneous effects of fructose on blood lipids in individuals with type 2 diabetes: systematic review and meta-analysis of experimental trials in humans. *Diabetes Care*. 2009 Oct;32(10):1930-7. [doi: 10.2337/dc09-0619](https://doi.org/10.2337/dc09-0619).



10. Wolever TM, Bolognesi C. Source and amount of carbohydrate affect postprandial glucose and insulin in normal subjects. *J Nutr.* 1996 Nov;126(11):2798-806. doi: [10.1093/jn/126.11.2798](https://doi.org/10.1093/jn/126.11.2798).
11. Meyer, M., Montero, L., Meckelmann, S.W. et al. Comparative study for analysis of carbohydrates in biological samples. *Anal Bioanal Chem* **414**, 2117–2130 (2022). doi: [10.1007/s00216-021-03845-z](https://doi.org/10.1007/s00216-021-03845-z)
12. Corradini, C., Cavazza, A., & Bignardi, C. (2012). High-Performance Anion-Exchange Chromatography Coupled with Pulsed Electrochemical Detection as a Powerful Tool to Evaluate Carbohydrates of Food Interest: Principles and Applications. *International Journal of Carbohydrate Chemistry*, 2012, 1–13. doi:[10.1155/2012/487564](https://doi.org/10.1155/2012/487564)
13. Cheng X, Kaplan LA. Simultaneous analyses of neutral carbohydrates and amino sugars in freshwaters with HPLC-PAD. *J Chromatogr Sci.* 2003 Sep;41(8):434-8. doi: [10.1093/chromsci/41.8.434](https://doi.org/10.1093/chromsci/41.8.434).