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A Circular Dichroism Analysis of Commercially Available Powdered Whey Protein Structure

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Abstract

Circular dichroism (CD) is a sensitive method that enables accurate detection of protein conformation, as well as structural changes in response to environmental factors such as processing and handling. Whey protein is generally considered the most nutritionally available protein powder source, but variance may exist even among whey proteins. Taking into account a variety of processing, storage and transport conditions, the purpose of this study is to explore whether those variations could result in detectable differences in CD assessed protein structure among different whey protein products available on the market. Secondary and tertiary CD as well as HT spectra of 10 various commercial dry whey products are presented, illustrating marked structural variations among the materials. This might point to the fact that external factors such as processing and/or handling techniques of whey products might have an impact on product characteristics and possibly their nutritional effects.

Keywords: Circular dichroism; High tension; Whey Protein; Stability; Nutrition; Powders

Introduction

Whey makes up 20% of the total protein content of milk and is sold as a nutritional supplement [1]. Whey is the liquid that remains after milk has been curdled, and the curds are strained [2]. It contains proteins, lactose, vitamins, minerals and traces of fat, and it is composed of five major proteins that make up 85% of whey protein-β-lactoglobulin, α-lactoglobulin, glycomacropeptide, proteose peptone 3, immunoglobulins and serum albumins. Almost all whey proteins exhibit significant bioactivities, and as such they have become of major interest in efforts to either reduce risk and/or prevent disease development [2]. Whey has also been recognized due to its potential as an adjunct to dietary plans and functional foods to regulate appetite and body weight, as well as metabolic processes [3]. As a supplement, it has been used for adjunctive therapy in cancer treatments as well as in the combat of infections, and its commercialization is vast as a number of companies have marketed it for a variety of diseases and conditions. Current whey products on the market include sweet and acid whey powders, reduced lactose whey, demineralized whey, whey protein concentrates, whey protein isolate, lactoferrin, lactoperoxidase, glycomacropeptide, dairy product solids and mineral-concentrated whey [1]. Furthermore, whey proteins also have several functional benefits in processing, which make them optimal as food additives for a variety of consumer groups [4]. These benefits include solubility, water-binding and viscosity, gelling, emulsification, whipping, foaming and aeration, dispersibility, edible film formation, antioxidant activity, adhesion property and heat-induced browning. Given the vast applicability and nutritional availability of whey protein products, it is safe to assume that prior to commercialization, they are exposed to rigorous and varied processing, handling and packaging processes (Figure 1) [5].

Rapid characterization of proteins is crucial not only in the fields of proteomics and genomics, but also in food science. Circular dichroism (CD) has been used since the 1960s, and it enables rapid assessment of secondary and tertiary structure as well as folding and binding of proteins. Briefly, it is defined as the difference in absorption of two components of polarized light of equal magnitude, one rotating counter-clockwise and the other clockwise [6,7]. Protein spectra depend on overall protein structure, and thus CD can be used to monitor any conformational changes that may have taken place as a result of environmental factors such as temperature, mutations, heat, denaturants or binding interactions [6-8]. Given that whey protein is considered the most nutritionally available protein powder source, variance among different whey products could be observed due to processing, handling and or transport. An investigation of the possible differences among whey protein products using CD has not been conducted to date. Here we tested whether the aforementioned factors that could affect protein structure do so by investigating structural differences among 10 commercially available powdered whey protein products via CD.

Objective

Using circular dichroism analysis, the purpose of this study was to investigate possible structural variations between commercially available powdered whey protein products.

Methods

A total of 10 whey protein products were analyzed, all of which are commercially available from GNC, Walmart and Amazon. All samples were diluted in 0.2 M Na_2HPO_4 buffer. Both concentrations of 0.2 mg/mL and 0.3 mg/mL were measured, and a superior signal was observed at 0.2 mg/mL which was used for all results. Results were analyzed using a one-way, repeated measure ANOVA using Prism 6 (GraphPad Software) achieving a $P<0.0001$.

A total of 8 measurements were taken per sample in CD, and HT modes simultaneously using a Jasco High throughput CD (J-1500), and the total data acquisition time per sample was ~2 hours. Data analysis

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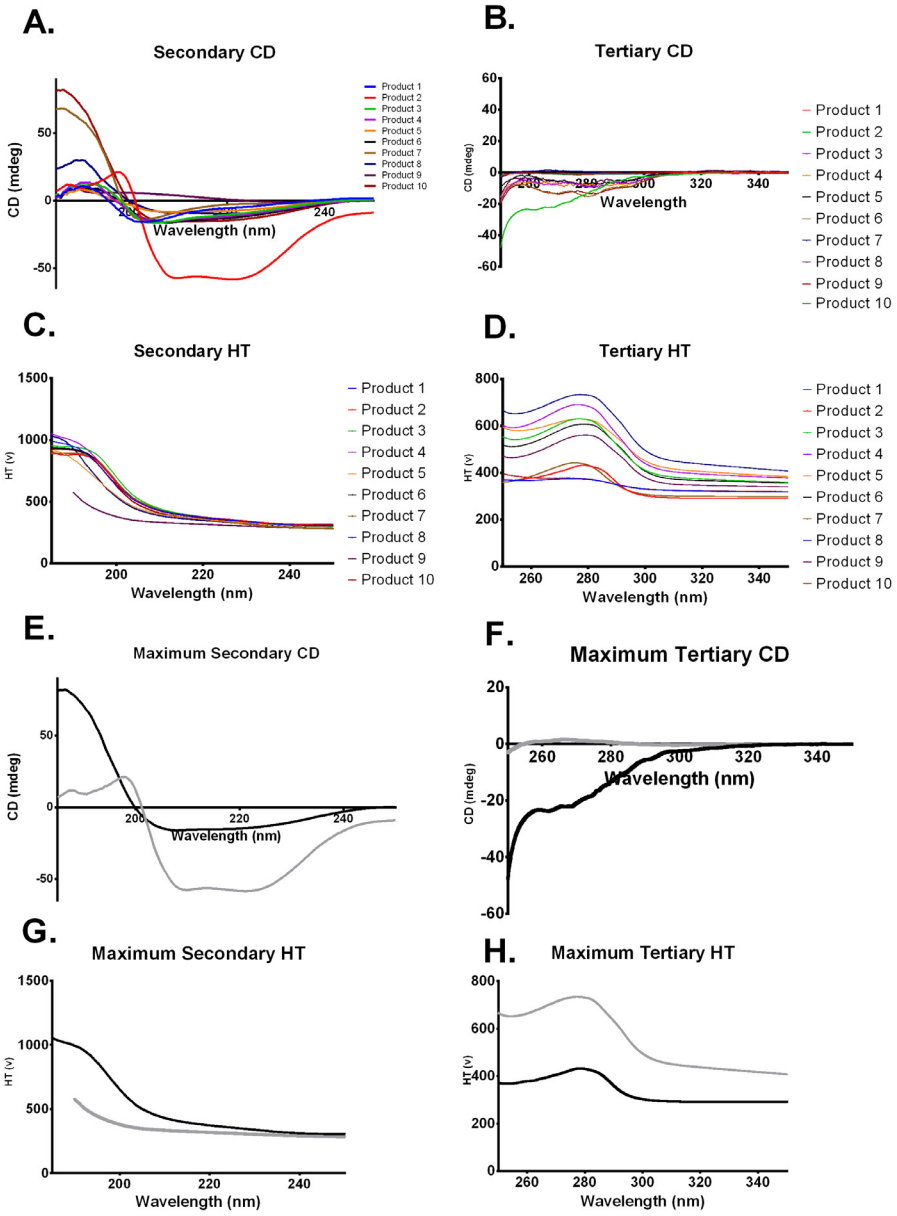

Figure 1: Secondary and Tertiary CD and High Tension (HT) spectra indicate large structural variations among the powdered whey products.
A. Secondary CD spectra are illustrated for the ten products that were analyzed.
B. Tertiary CD spectra are illustrated for the ten products that were analyzed.
C. Secondary HT spectra are illustrated for the ten products that were analyzed.
D. Tertiary HT spectra are illustrated for the ten products that were analyzed.
E. Secondary CD are illustrated for the two products that demonstrated the highest variance.
F. Tertiary CD are illustrated for the two products that demonstrated the highest variance.
G. Secondary HT are illustrated for the two products that demonstrated the highest variance.
H. Tertiary HT are illustrated for the two products that demonstrated the highest variance.

was performed using Spectra Analysis and the Multivariate Secondary Structure Estimation software.

The secondary, tertiary and HTCD parameters are listed below.

Secondary structure measurement parameters:

Cell: 1 mm pathlength

Wavelength range: 190-250 nm

Data Pitch: 0.1 nm

Scan Speed: 50 nm/min

DIT: 4 seconds

Accumulations: 5

Tertiary structure measurement parameters:

Cell: 1 cm pathlength

Wavelength range: 250-350 nm

Data Pitch: 0.1 nm

Scan Speed: 100 nm/min

DIT: 4 seconds

Accumulations: 3

HTCD parameters:

Air Volume to Load: 1015 mL

Sample Volume: 100 mL

Wash Cycle: Detergent 200 mL, Water 200 mL, Acetone 200 mL x 2

Dry Time: 180 seconds

Results & Discussion

To quantify the variance between whey protein powders available on the market, 10 commercially available whey protein products (available from from GNC, Walmart and Amazon) were used as source material for the study. The whey protein powder products were diluted to 0.2 mg/mL in 0.2 M Na_2HPO_4 buffer and analyzed using circular dichroism (CD) spectroscopy. We observed the secondary and tertiary structures of the whey proteins exhibited significant structural variations, as evident in the products' CD spectra (Figure 1A-D). The two traces that have the largest difference in ellipticity and thus highlight the highest structural variation are illustrated in Figure 1E-H. Furthermore, the vast differences observed in tertiary structure, whereby only one product has a distinguished peak in the near-UV region, point to potential protein stability discrepancies (Figure 1B).

The structural properties of proteins are influenced by environmental factors, such as pH, temperature and salts, all of which result in characteristic functional properties, such as gelation, foaming and emulsifying activity that are useful in food applications. Furthermore, a variety of storage and transport conditions are implemented in the handling of commercialized whey protein products [9]. Whey protein products are also exposed to various processing techniques and treatments such as evaporation and spray drying which denature whey proteins, all of which could manifest themselves as differences in structure. Furthermore, heat treatments applied to milk and whey prior to any other treatments have also been proposed as factors for structural variety [10]. Here we show that whey proteins from different manufacturers have large structural variations and thereby highlight the effects of the aforementioned environmental factors on secondary and tertiary product structure [11]. Circular dichroism methods to date have been implemented in investigations in which effects of changes in external factors on protein structure have been directly measured, but using this technique as means to directly monitor food quality and consistency could minimize variations and thereby standardize commercially available products.

Conclusions

In this study, we analyzed 10 whey protein products that were commercially available from GNC, Walmart and Amazon. We detected large structural variations both in the secondary and tertiary protein structures among the products. We speculate that this is possibly due to thermal denaturation from various whey protein processing techniques (spray drying), storage, or transportation conditions by the manufacturer and retailer or labeling discrepancies. This is in line with our expectations, as previous studies have demonstrated the impact of pH or temperature on overall protein structure as detected via CD. In conclusion, structural differences between several commercially available whey protein products were detected, and this is potentially due to the processing and handling prior to distribution. Our findings suggest the use of the CD analysis method as an assessment of food processing and handling effects on substances like dry protein powder.

Limitations

While the protein source materials used in this investigation are commonly found in commercial use, there could be variations within the individual materials used. Further studies are needed to examine the structural variances between individual commercial products over time.

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