The Effect of Atmospheric Plasma on Cold Thermal Stability of Powdered Whey Protein Isolate

Michael D Birnbaum

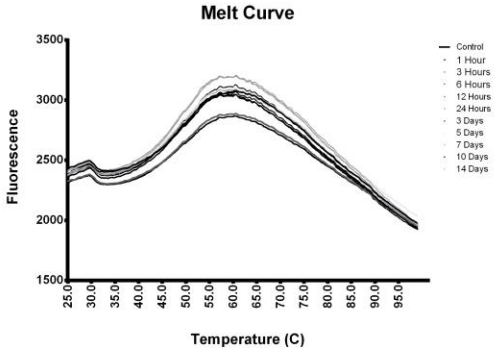
Department of Molecular and Cellular Pharmacology, University of Miami, United States
Email: MBirnbaum@med.miami.edu

Abstract:

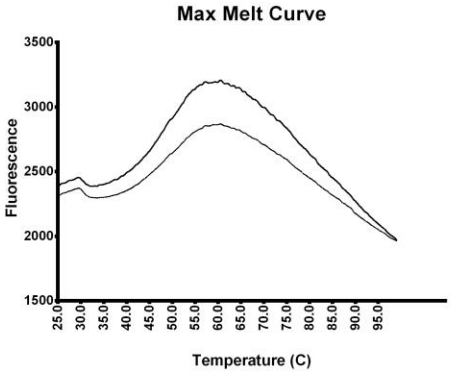
Thermostability is the capacity of a material to withstand irreversible change in its structure by resisting extreme external factors such as high relative temperature. Extensive efforts toward making protein-based biological substances such as vaccines thermally stable have been made by implementing treatments such as lyophilisation, biomineralization, and encapsulation in sugar glass and organic polymers. These substances have a typically short shelf life, as they denature and degrade at room temperature over time. Furthermore, efficient storage and distribution relies on continuous refrigeration in order to preserve protein stability. However, this is costly and not always effective, as any disturbance in storage and distribution conditions may lead to rapid loss of effectiveness and potency. Whey protein isolate is used in a wide variety of food applications and is at risk of exposure to freezing temperatures during its transportation, which in turn could affect its stability as well as chemical and physical structure. This study examines the effects of plasma surface modification (PSM) on whey protein thermostability. Here we report on structural changes in commercially available whey protein exposed to cold thermal conditions, as reported by the Protein Thermal Shift Assay (PTSA). An improvement of 48% in protein thermal stability was observed upon treatment with PSM, suggesting that PSM may reduce damage caused by temperature fluctuations.

Figures:

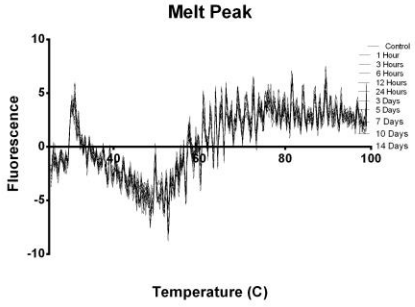
A.


Melt Curve
Fluorescence
Temperature (C)
Control
1 Hour
3 Hours
6 Hours
12 Hours
24 Hours
3 Days
5 Days
7 Days
10 Days
14 Days

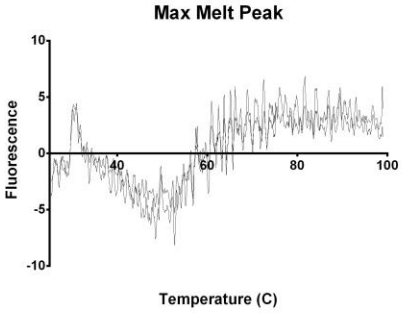
B.


Max Melt Curve
Fluorescence
Temperature (C)

C.


Melt Peak
Fluorescence
Temperature (C)
Control
1 Hour
3 Hours
6 Hours
12 Hours
24 Hours
3 Days
5 Days
7 Days
10 Days
14 Days

D.


Max Melt Peak
Fluorescence
Temperature (C)

E.

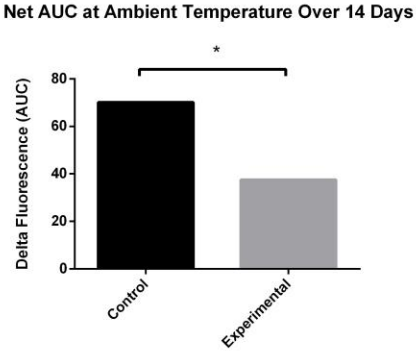

Net AUC at Ambient Temperature Over 14 Days
Delta Fluorescence (AUC)
*
Control
Experimental

Figure Legend:

Figure A: Comparison of Melt Curve measurements of whey protein samples cold-treated for 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 3 days, 5 days, 7 days, 10 days and 14 days as compared to untreated control.

Figure B: Comparison of Melt Curve measurements of 14 day cold-treated whey protein and PSM-treated whey protein as compared to control.

Figure C: Comparison of Melt Peak measurements of whey protein samples cold-treated for 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, 3 days, 5 days, 7 days, 10 days and 14 days as compared to control.

Figure D: Comparison of Melt Peak measurements of 14 day cold-treated whey protein and PSM-treated whey protein as compared to control.

Figure E: Comparison of Melt Curve, Area Under Curve of untreated and plasma-treated whey protein powders measured at Ambient Temperature (22 Degrees Celsius) over 14 days. Asterisk (*) indicates 48% reduction in melt curve alteration by cold temperature, $p<0.001$.

Introduction:

Proteins have ideal temperature ranges required to maintain structural integrity, which are defined by their sequence and folding. Organisms express and adapt proteins to function most efficiently in the protein's environmental temperature.[1] However, proteins outside of their evolutionarily adapted environment can suffer from reduced stability. Understanding a protein's behavior under various conditions is pivotal in efforts to preserve its efficacy and protect it from environments that alter its functionality. Whey proteins are vastly applicable not just in the food and nutrition industries, but also in biomedicine as adjunct therapies. Given their broad utilization spectrum, studying their folding properties and structure is essential to understanding their functionality and how to preserve it. Previously, physical and chemical changes have been observed in whey protein as results of elevated temperatures and humidity.[2] Furthermore, whey protein's functionality as a food protein is highly affected by its processing conditions and modifications.[3] This is also true for processing, transport and handling of whey protein, which expose the product to several different environments both intentionally and unintentionally, affecting its final functionality. It is therefore essential to better understand the physicochemical and structural properties of whey proteins in response to stress, and

to identify and optimize methods by which whey protein can be processed and transported without damage.

Recent developments in protein processing have identified plasma surface-modification (PSM) as a new and interesting protein treatment. Plasma is a gas that has been charged with electrons in both the negative and positive state and as such is characterized by both high conductivity as well as high internal energy.[4] Plasma treatment is said to be initiated when plasma ions come in contact with a particular surface. As such PSM is a process whereby plasma is applied to the surface of a material in order to alter its chemical properties, such as surface area and hydrophilicity.[4] Briefly, plasma treatment is performed in a vacuum chamber with controllable pressure and temperature.[5] Different processed gases may be used during plasma treatment, including atmospheric plasma, generated by using processed atmospheric air.[6] Ultimately, PSM enables selective alterations of protein structure with granular control by altering the reaction parameters of the plasma exposure. However, the effect of atmospheric plasma on the stability of organic protein powders during processing and subsequent temperature-variable transportation has not yet been studied.

For the purposes of this study, cold-induced degradation and PSM-induced protein changes are measured via the Protein Thermal Shift Assay (PTSA). PTSA is used for screening small molecules in the drug development process with the capability of high-throughput analysis based on very small sample quantities in order to identify protein structure, stability, ligands, mutations, modifications, and buffer conditions. Briefly, ramping up temperature results in a 'thermal shift,' wherein heat denaturing and other changes in conformation expose hydrophobic pockets that bind to the fluorescent PTSA dye. Quantifying and comparing this thermal shift provides an effective evaluation method for the stabilization of the protein under various conditions.[7] PTSA has become known as a popular protein melt analysis method, as other protein melt screening processes tend to be inefficient and/or expensive. It has also been a popular small molecule screening method in the drug development process due to its high-throughput and accurate output identifying protein structural changes. The method also relies on very small amounts of starting material, which make it more economical and material friendly.

Objective:

The objective of this study is to investigate the thermal stability of dry PSM-treated and non-treated powdered whey protein isolate under cold thermal conditions using a protein thermal shift assay.

Results & Discussion:

This investigation specifically assessed the impact of cold storage and plasma-surface modification on dry powdered whey protein isolate. Proprietary PSM technology (Ingredient Optimized™, Plasma Nutrition, USA) was used to effectively mediate the exposure of organic dry powder materials to plasma to enact multiple changes, including protein structure alterations. For this study, we obtained a commercially available dry powder whey protein isolate as source material. This base material served as the untreated control for the experiment. A Protein Thermal Shift assay (PTSA) was conducted on samples of whey protein isolate exposed to various cold storage conditions, atmospheric plasma, and compared to untreated pristine samples.

Both the Protein Thermal Shift (PTS) melt curves (Figure 1 A,B) as well as melt peaks (Figure 1 C,D) indicate effects of cold storage on the dry powdered whey isolate. Structural changes are observed across all cold exposures (Figure 1 A,C). As expected, the largest differences were observed between untreated and longest cold treatment. The untreated and two-week cold storage samples were then compared to the melt curve and melt peak of a PSM-treated protein that was also subjected to two weeks of cold storage (Figure 1 B,D). As seen in the figure, there is a significant rescue of the structural damage caused by cold storage in the PSM sample. All described results were statistically significant ($p<0.0001$). A total protein structure stability change due to PSM was obtained by calculating the area under the curve of the PTSA melt peaks of the PSM and non-plasma-treated 2-week cold storage samples, and comparing to the untreated control. The change in melt curves showed that PSM-treated protein suffered 48% less change in its structure and stability compared to the non-plasma-treated control after two weeks of cold storage (Figure 1E).

Changes in protein stability as observed by folding and degradation assays such as PTSA are well established markers of a protein's structure and function[8]. Understanding a protein's structure also helps in efforts to preserve that structure as degradations of protein-based products lead to a shorter shelf life and, in most severe cases, a loss of that product's function. This is particularly detrimental in proteins that are used for medical purposes such as vaccines, whose additives are carefully chosen to withstand storage conditions as means to preserve their functionality.[9] This report demonstrates that PSM is an effective method for protecting against changes in food protein structure due to external storage factors such as exposure to cold temperatures. We also demonstrate that a simple and high-throughput PTSA is suitable for detecting these structural changes. While PTSA is most widely adapted for drug discovery efforts and ligand-binding studies,our results suggest additional practical uses for PTSA and PSM Used in conjunction, PTSA and PSM may lead to improvements in the analysis and development of commercially available protein products, both the food and nutrition industries.

Conclusions:

Commercially available whey protein isolate has a low cold thermal stability which may negatively affect its applicability in the food and nutrition sectors. Exposure to cold temperatures for as little as one hour was enough to cause measurable changes in protein stability, and loss of stability continued to accumulate over the 14 days examined. Here we report that exposing whey protein isolate to atmospheric plasma improves 14-day cold thermal stability by 48%. These results suggest plasma treatment as a novel and effective means of promoting protein thermal stability and preservation.

Methods:

A commercially available dry whey protein isolate powder was used for the study. Within that batch, treated powder was used as source material and the untreated experimental control. Source material was exposed to atmospheric plasma conditions using proprietary PSM technology (Ingredient Optimized ™, Plasma Nutrition, USA) technique described above. All samples were stored in a commercial freezer emulating "cold-chain" commercial storage between 10F and 20F for the following durations: 1h, 3h, 6h, 12h, 24h, 3 days, 5 days, 7 days, 10 days and 14 days. Control powder from the same batch was left untreated and unexposed to cold.

Protein samples were diluted in phosphate buffer and Applied Biosystems Protein Thermal Shift dye was added according to the manufacturer's protocol. Each sample was run alongside two control samples to ensure accuracy (buffer only, buffer + control protein and buffer + control protein + control ligand). Four replicates of each protein melt reaction were tested to ensure statistically significant results. All protein samples were stored at a constant temperature between 10F and 20F for the selected duration. The melt assay was run on a ThermoFisher Quantstudio real-time PCR system. Reaction results were individually and manually verified by the technician to make sure no pipetting, setup or assignment errors occurred. Melt curve and melt peak data were obtained via Applied Biosystems Protein Thermal Shift software.

Statistical analysis was conducted via Prism 6 (GraphPad Software) using a one-way matched ANOVA.

Limitations:

This study investigated the application of PSM on only one source of dry powdered whey protein isolate. While the whey protein isolate source material used in this investigation is commonly found in commercial use, there may be variations between the material used and other market-available whey protein isolate powders. Further studies are needed to examine the effect of PSM on a range of dry powder whey protein isolate sources.

Funding Statement
This work was financially supported by Plasma Nutrition.

Ethics Statement

All experiments were performed in vitro and did not involve the use of any living subject. No fraudulence is committed in performing these experiments or during processing of the data.

Sources

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