How Advances in Analytical Instrument Technology Heighten Efficiencies for the Freeze Drying Industry
An Application of the Lyotherm3 and Lyostat5 on Freeze Drying Fruit Juice for Easy Transport and Long-Term Storage

Rationale

Lyophilisation is a dehydration process which has been used throughout history as a means of preventing deterioration of perishable materials (the Incas stored potatoes at high altitudes where low pressures and temperatures dried them preventing them from spoiling) [1]. First mentioned in literature by Altman in 1890, it was developed by d'Arsonval in 1906 and later by Harris and Shackell for Freeze Drying live rabies virus for the first freeze-dried vaccine before being fully developed as a process for long term storage of blood cells towards the end of World War Two.

Freeze drying is designed to remove more than 99% of the bulk water content of a product with the aim to increase its stability and maintaining high activity for between 6 months and 10 years. Material is frozen to a pre-set limit temperature and held to ensure the temperature equilibrates. Once the material has reached its limit temperature, a vacuum is applied to start the sublimation process.

There are two phases to the drying process – Primary and Secondary Drying. Typically during Primary Drying a pressure less than 2/3 of the literature value of the vapour pressure of ice is selected as a pressure set-point and this is monitored throughout the drying cycle while the bulk water is removed. Bulk water makes up between 95 and 99% of the total water with the remaining water content made up of adsorbed moisture. The chemically bound (adsorbed) moisture is removed during the secondary drying phase where the temperature is increased to a point where the material is unlikely to melt and therefore relatively stable and the pressure reduced to a minimum. The length of secondary drying is proportional to the desired moisture content (how dry the final product needs to be) so the secondary drying process can be quite long and slow.

During the primary drying process higher temperatures results in more efficient cycles. For every 1 degree higher a product results in a 13% saving in efficiency [2] however freeze drying at temperatures too high can result in large variabilities in product stability and appearance. This can typically be alleviated through trial and error however this is a lengthy process with many iterations.

Analytical instrumentation can be used to determine the temperatures critical to the product so that it can be freeze dried without risking damage to the product. Freeze Drying Microscopy is used to visually observe the breakdown of the product during freeze drying while Thermal Analyses are used to observe any changes in the structure (such as exothermic or endothermic changes) of the product in comparison to a reference material. Knowledge of these temperatures provides an indication of the limit of the primary drying temperature so that the risk of damaging the product is reduced.

During the sublimation process the temperature of the product reduces as the ice is removed to below that of the set-point temperature: this is known as sublimation cooling. The amount of sublimation cooling can be accounted for during freeze drying so that although the shelf temperature may be higher than the critical temperature of the product the product temperature still remains safely below this point.

Freeze Drying is also widely used in food preservation and storage. Traditionally the way of preserving fruit juice is through Ultra-Heat Treatment (UHT) to sterilise the product and eliminating any contamination however, the cost of shipping dilute liquids remains high due to the high water content of the product. The costs associated with shipping and storage before final packaging has been reduced through the development of concentration methods although there are still risks to the product through thermal damage and cross contamination.

In this regard, a study was carried out using PepsiCo's Apple, Pineapple and Kiwi 'Green Machine' smoothie as it contains all natural ingredients as well as supplements for Vitamin A and C.

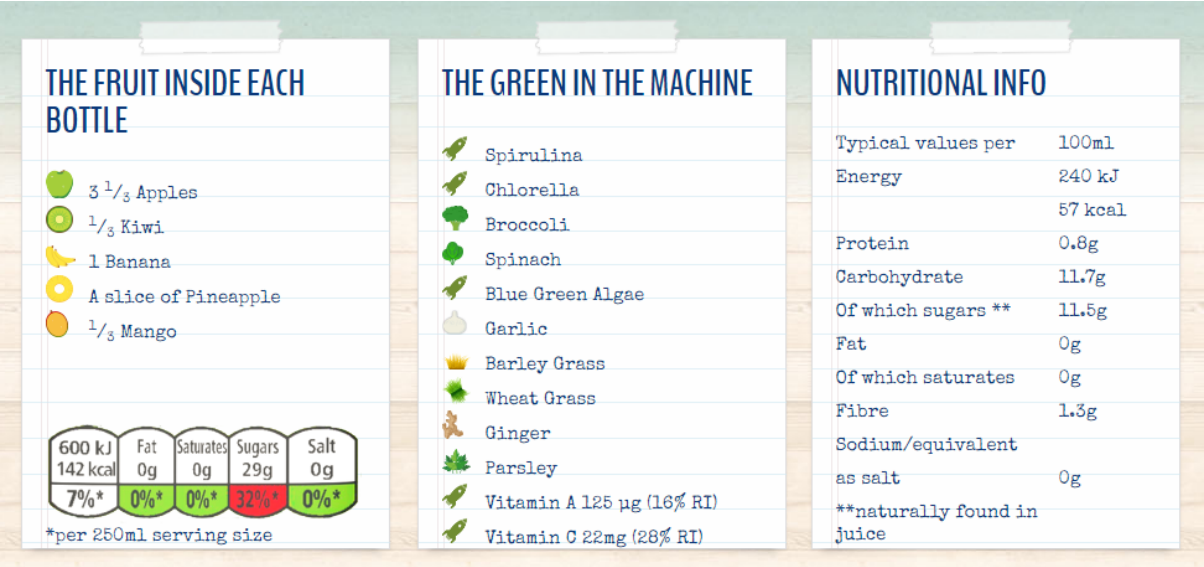

Figure 1: Nutritional Values and ingredients for 'Green Machine' Smoothie.

Methods

A 2µL sample of Fruit Juice was analysed using a Lyostat5 Freeze Drying Microscope (Biopharma Process Systems Ltd, Winchester, 2016). The sample was loaded into the analysis chamber as per FIGURE X. The temperature was reduced to -40°C at a rate of 20°C/minute. Once the stage reached -40°C the sample was held for 5 minutes to equilibrate the temperature before the vacuum was applied. The pressure was reduced to 3µBar and maintained to dry the product and, once a good amount of dried material had be formed the temperature was increased at a rate of 1°C/minute until the collapse of the material occurred. The onset of collapse is taken as the critical temperature (i.e. before any breakdown of structure is observed).

In addition, 6mL sample was analysed using a Lyotherm3 Differential Thermal and Impedance Analyser (Biopharma Process Systems Ltd, Winchester, 2016). The 3mL sample was placed in a sample cuvette and a Pt100 probe was inserted into the sample, an additional 3mL was placed in a second cuvette and an Impedance was placed into the sample. Finally 3mL of reference material (Analar water) was placed into a third cuvette and a Pt100 probe was inserted into the reference. The probes were secured in place with a clamp and the sample assembly was lowered into 1.5L liquid Nitrogen. The samples and reference were cooled to below -100°C once below this point heat was applied (48W, 1A) until the samples and reference were heated to above 0°C. The temperatures of the sample and reference and the Impedance of the sample were plotted on heating over the temperature range.

Results

The onset of collapse temperature obtained by the Lyostat for the Fruit Juice was –37.0°C, this became more apparent as the temperature increased (--33.6°C) before the total collapse of the product was observed (-22.0°C).

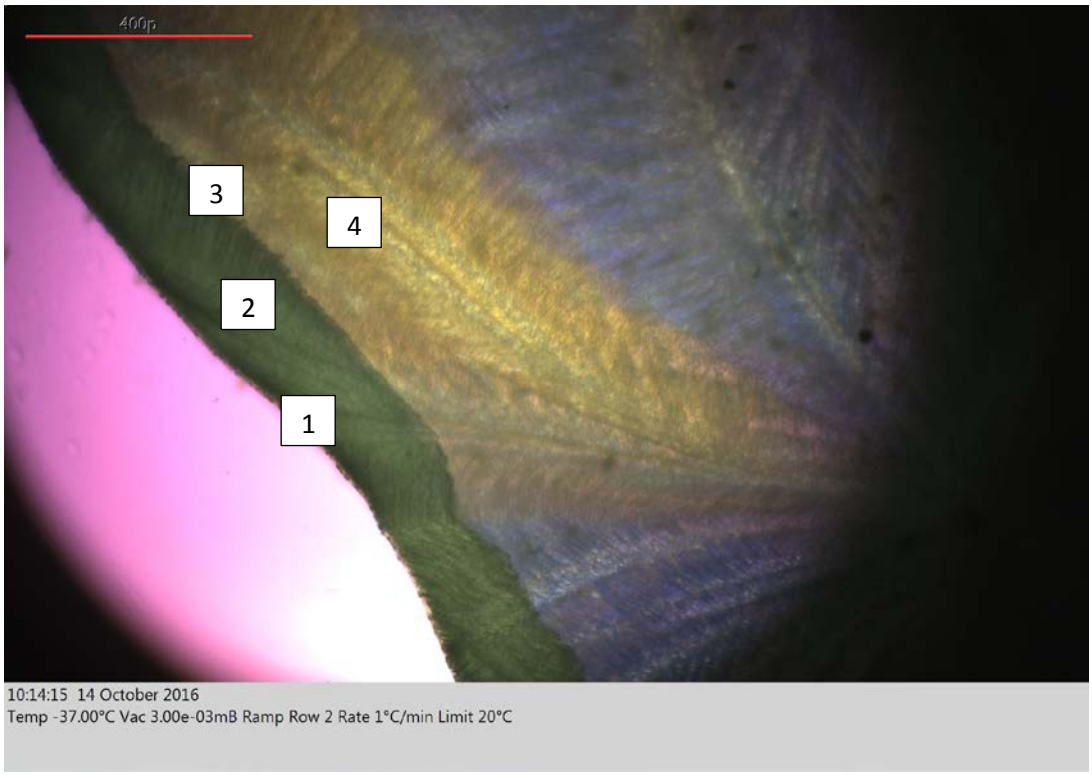

Figure 2: Onset of collapse of the Fruit Juice.

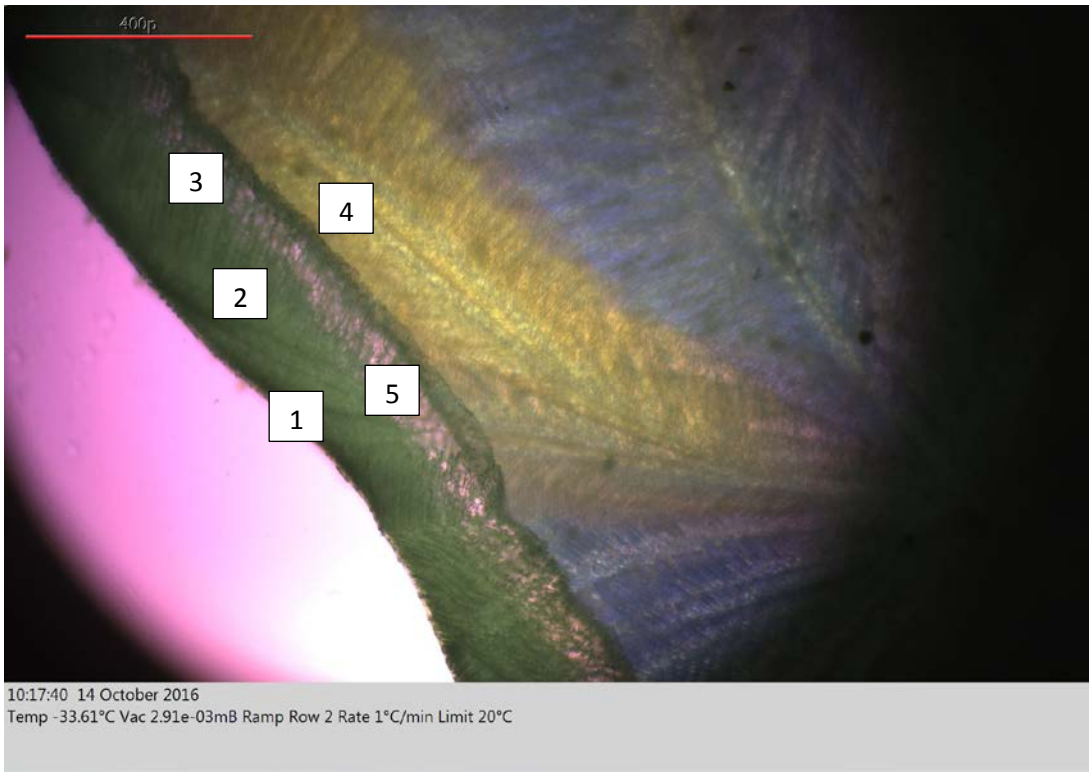

Figure 3: More apparent collapse of the Fruit Juice.

The Lyotherm analysis showed transitions both on the DTA and Impedance lines which have been summarised in the table below.

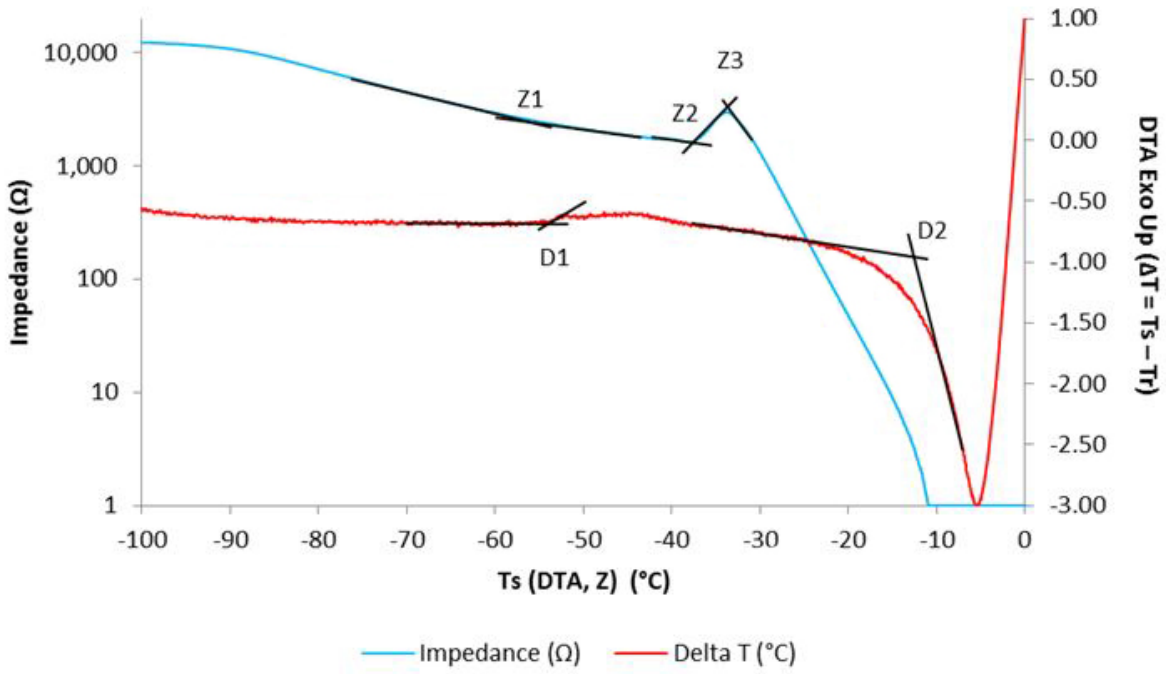

Figure 4: Graphical Output for Lyotherm3 analysis of Fruit Juice.

Table 1: Lyotherm3 Results

Analysis	Event	Temperature	Description
Impedance	Z1	-56.2	Decrease in the downward of the gradient of the Impedance line indicating stabilisation of the material.
	Z2 to Z3	-37.6 to -34.1	Increase in impedance indicative of a stabilisation within the frozen material.
DTA	D1	-54.5	Onset of Crystallisation of one or more components within the formulation.
	D2	-13.8	Onset of significant endotherm indicating bulk solvent melt of the sample

Discussion

Between –37.6°C and -34.1°C an increase in the impedance is observed, suggesting that a stabilisation or rearrangement of the frozen structure is occurring at this point. This event is coupled with an extended exothermic event exhibited in the DTA between -54.5°C and -37.2°C, which also indicates an increase in order within the frozen structure. This event could be associated with the crystallisation of one of the amorphous components of the formulation. A considerable decrease in the impedance is observed, beginning at -34.1°C, signifying the onset of substantial softening of the material and increasing molecular mobility within the frozen structure. The impedance thereafter continues to fall rapidly until –11.1°C where minimum impedance is reached. This is allied with an endothermic event observed in the DTA at -13.8°C, which is also indicative of a softening event.

The FDM analysis shows the progression of the sublimation front [3] as it moves from the edge of the sample [1] and continues to dry [2] in the direction of the undried material [4]. This data corresponds with the data obtained from FDM analysis, which shows that the onset of collapse of the drying material [4] begins at –37.0°C and progresses to total collapse as the temperature is increased [5]. An increase in impedance is also observed between –37.6°C and –34.1°C, which is most likely the consequence of the complex blend of organic molecules present in the formulation. The large endothermic peak observed at -13.8°C is associated with the melting of the bulk ice structure present, and is commonly known as the 'Ice melt endotherm'.

Conclusions

Results from Impedance and DTA analysis on the Lyotherm, both corroborated the observations from Lyostat analysis and provided a more complete picture of the behaviour of the frozen material. Experience would suggest that products that exhibit a 'collapse zone', characteristically display some evidence of collapse when material has been freeze-dried with product temperatures maintained within the collapse zone. However, for some products, sufficient structure is maintained to produce a cosmetically acceptable dried cake.

In order to achieve complete maintenance of structure, the product temperature should be ideally maintained below its respective lower collapse zone limit throughout primary drying, in order to prevent product collapse.

For additional product safety and quality, it is recommended that, in accordance with common current practice, freeze-drying cycles are developed for the product that incorporates a "safety margin" of between 2°C and 7°C with regard to product temperature, in order to allow for slight variations that may be experienced during scale-up, technology transfer and the use of different freeze-dryers.

Thus, according to current common practice, it is recommended that the formulations are frozen below the temperatures summarised (actual product temperature) and maintained below these temperatures until the end of the primary drying process.

If a solution is processed with product temperature maintained within the collapse zone range in primary drying, the activity of the final product should be analysed even if the product is cosmetically acceptable.

References

1) M. Willard, Potato processing: Past, present and future, American Potato Journal May 1993, Volume 70, Issue 5, 405–418.
2) M. J. Pikal. Freeze-drying of proteins. Part I: process design, BioPharm 3:18–28 (1990).