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

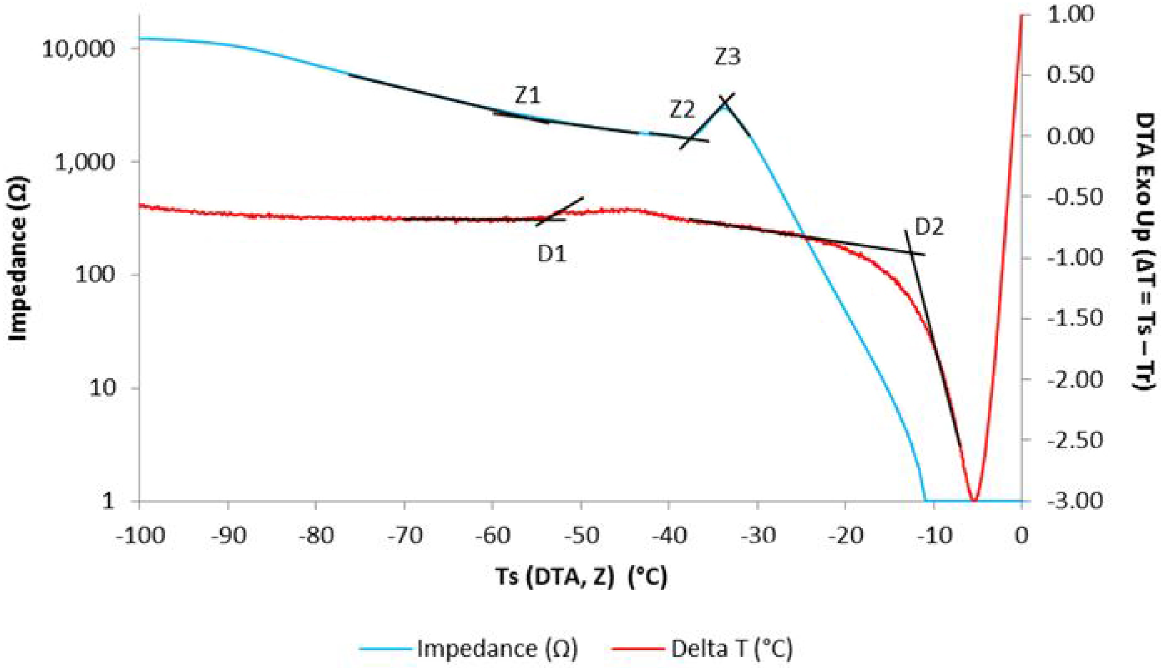

Figure 4: Graphical Output for Lyotherm3 analysis of Fruit Juice.

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.

From figures 2 & 3 the Lyostat5 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 Lyostat5 FDM analysis, which shows that the onset of collapse of the drying material [4] begins at –37.0°C and progresses to total collapse [5] as the temperature is increased. An increase in impedance is also observed between –37.6°C and –34.1°C (Figure 4) 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 Lyotherm3, both corroborated the observations from Lyostat5 FDM 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.

Ref

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).