freeze-dried using three different freeze-drying protocols. These protocols were chosen to produce a collapsed cake, a micro-collapsed cake, and a noncollapsed cake. This investigation aims to test the impact of the micro-collapsed cake on the protein stability.

The three freeze-drying cycles were selected to produce cakes with different physical properties. The first, "no collapse" (NC), was a very gentle cycle, in which the samples were annealed to allow crystallization of the glycine eutectic to occur, and the primary drying temperature was very low. The second cycle, "micro-collapse" (MC), also included an annealing step, but the primary drying temperature was much higher. The third cycle, "collapse" (C), did not include an annealing step, and the temperature during primary drying was also kept low to inhibit the crystallization of the glycine eutectic. Following freeze-drying, the products were placed in stability chambers at 5°C, 25°C, and 40°C to assess their long-term stability during storage at each of these temperatures. Sample vials were removed from the stability chambers periodically and assayed for protein activity, moisture content, and aggregate by SEC-HPLC.

Figure 16 is a photograph of samples freeze-dried by each of the three methods, and shows the level of physical, macroscopic collapse that typically resulted from using the collapse freeze-drying method.

Scanning electron microscopy (SEM) showed that the C cakes are significantly different on a microscopic scale from the other cakes, as shown in Figure 17. The magnification of these pictures is 760×. The C cake shows much less structure than is observed for the NC or MC cakes, which at this magnification are indistinguishable. No evidence was detected by SEM for any level of collapse in the MC samples.

Figure 18 shows the average protein activity measurements for each freeze-drying protocol up to the 18-month time point of the stability study. This plot shows data from each of the storage temperatures, 5°C, 25°C, and 40°C, as well as for liquid samples that were taken immediately prior to freeze-drying, and zero time point (T_0) samples taken immediately after freeze-drying. The

FIGURE 16 Photograph of noncollapsed (NC), micro-collapsed (MC), and collapsed (C) α-amylase samples after freeze-drying.