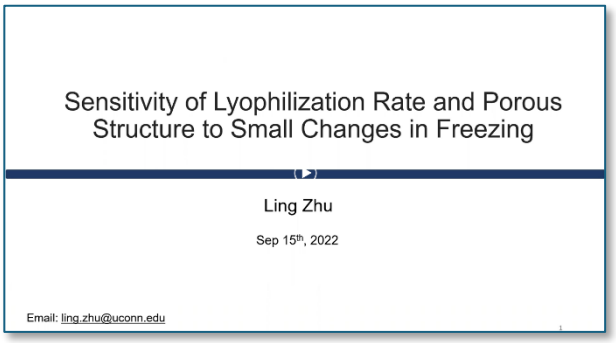
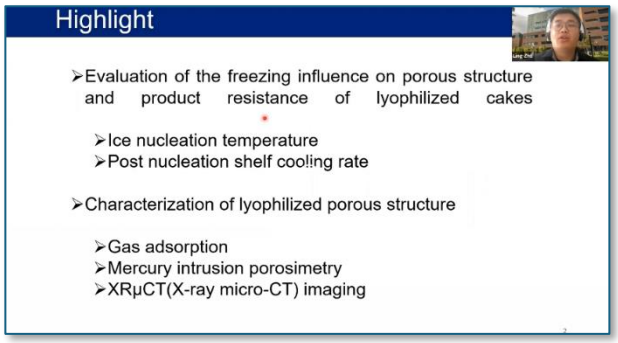
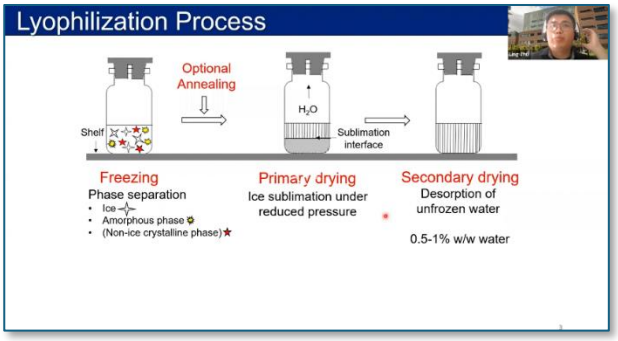
Sensitivity of Lyophilization Rate and Porous Structure to Small Changes in Freezing (webinar transcription) presented by Ling Zhu, Graduate assistant at University of Connecticut.

In today's webinar we will learn about the following parts. First, the evaluation of the freezing influence on porous structure and the product resistance of lyophilized cakes. In this part, the freezing influence will be discussed based on two factors, the ice nucleation temperature, and the post-nucleation shelf cooling rate. In the next part we will learn about the characterization of lyophilized porous structure. In this part, three techniques will be discussed. First the gas adsorption and the mercury intrusion porosimetry and last the X-ray micro-CT imaging.





Before coming to the research part, I will give a brief introduction on the lyophilization foundation process. As shown in this slide, the lyophilization process is divided into three steps, freezing, the primary drying stage, and then secondary drying stage. During the lyophilization, the sample vials are in contact with the shelf and the shelf has a temperature control. In the freeing step, the shelf temperature decreases and the water in the solution converts to ice. And the left solute and some unfiltered water in the solution will form into a freeze concentrate and there yearly in the state of amorphous. Some non-ice substance could form crystalline phase two. In the freezing, sometimes annealing is applied. In annealing the shelf temperature was raised in a short period of time and this will alter the formation of ice.

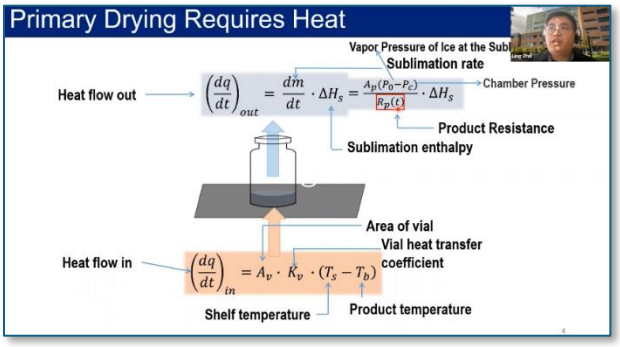


Primary drying involves the removal of all the ice by sublimation under reduced pressure. In this step, the shelf temperature is raised to increase the sublimation rate of the ice. When the primary drying is finished, the shelf temperature will be elevated even higher, and the second drying starts.

The second drying involves the removal of unfiltered water from the freeze-concentrate by desorption. And secondary drying is also carried out under reduced pressure. When the secondary drying is finished, usually around 0.5% to 1% weight by weight water will remain in the final product.

Among all the three steps, the primary drying is the most time-consuming step. A shorter period of time of primary drying will significantly improve the efficiency of lyophilization. However, it cannot simply be achieved by adding heat to increase the sublimation rate because to ensure the product quality, appropriate heat must be supplied to maintain the product temperature below the critical

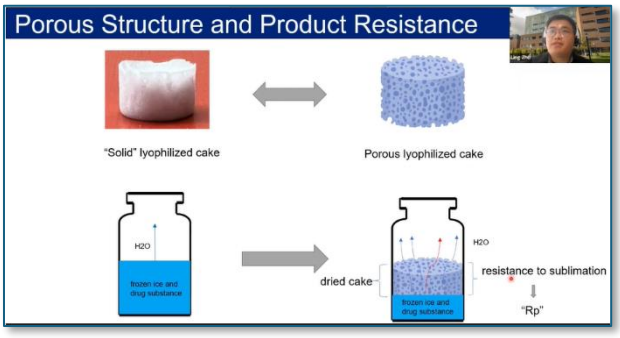
temperature. So, it is necessary to learn how to improve the efficiency of the primary drying. In order to do that, it is important to understand the role of heat in primary drying.



Heat flow is critical doing primary drying. It involves two parts, the heat flow in part and the heat flow out part. And as shown in this slide, a mathematical model was used to expand the treat transfer process in the primary drying. Show in this slide at the bottom part of the slide, it shows the heat flow in part. In this part, the sample vial accepts the heat from bottom shelf and the shelf temperature is controlled. From this equation, we can see that the differences between the shelf temperature and the product temperature determine the magnitude of the heat flow in flux.

Meanwhile, the Kv in this equation, the vial heat transfer coefficient also influenced the transfer of the heat. From the equation we can see that the larger the vial heat transfer coefficient is, the faster the heat will be transferred, and the heat transfer coefficient is influenced by several factors such as the gas conduction and the radiation heat. When the heat flows into the sample vial, the ice inside the vial absorbs the heat and starts to sublimate. Under the sublimation of the water vapor or the removal of the ice will cause the heat flow out of the vial. This can be described by the heat flow out part on the top of the part of the slides.

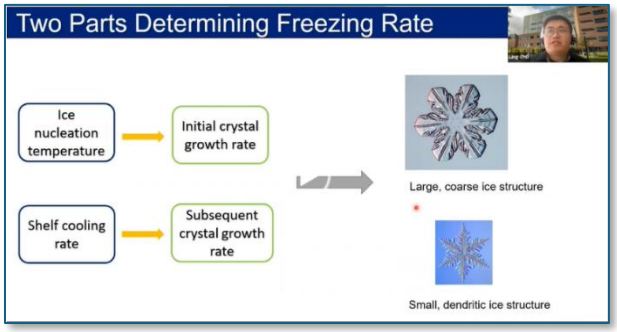
We can see from this equation, the driving force that takes water vapor out is determined by two factors. First, the difference between the chamber pressure and the vapor pressure of the ice at the sublimation interface. And also, it is influenced by the Rp, the product resistance. The product resistance describes how easily the water molecules can escape the vial during primary drying. The higher the resistance is, the slower the heat will flow out of the vials. And from these two models we can see that any factors that influence the heat transfer coefficient and the product resistance could influence the primary drying speed. And for today's webinar we will focus more on how the resistance influences the primary drying rate.



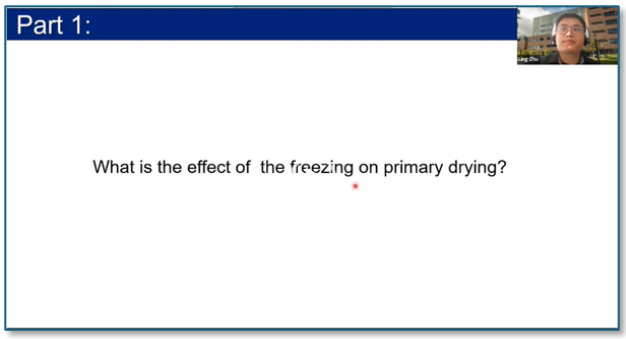
The resistance to sublimation is the structure-based properties. As shown in this slide we try to look into the product structure to figure out the underlying reason that controls the process. As shown in this picture, it is a typical lyophilized cake that formed in the lyophilization. It is shown as a solid, it is a cake full of various pores as shown in the purple color in the red part. These pores are formed due to the leaving of water during the drying part. A typical lyophilization cake often has around 75 to 95 pores. And as shown in the slides, when the sublimation starts, the water starts to leave from the top of the cake and as the process proceeds the water removed from the upper part and leaves the pores in the dried cakes, which is

shown in the purple color. And the water molecules in the lower part have to pass through these pores to escape the file when they start to sublimate.

This causes resistance to the sublimation below the dried cake, which is shown as the Rp here. As a result, we can see that the size of the pores influences the value of the resistance. This gives us the idea that by modifying the porous structures, the product resistance can be reduced and shorten the primary drying. Since freezing can impact the porous structures, we try to explore the influence of the freeing rate on the product resistance and the primary drying.

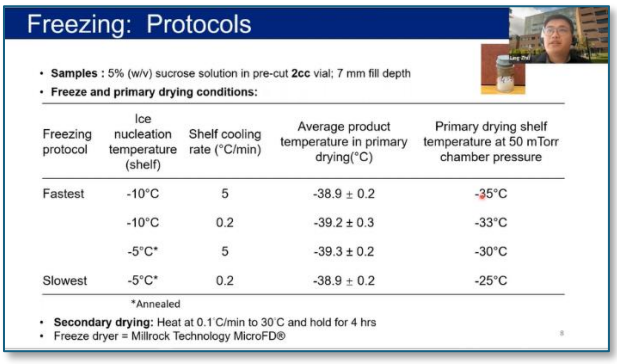


And as shown on this slide, the freezing rate is influenced by two parts, the ice nucleation temperature, and the shelf cooling rate. The ice nucleation temperature influenced the initial crystal growth rate and the extent of the ice formed. While the shelf cooling rate influenced the subsequent crystal growth rate after ice nucleation. And generally, a slow freezing rate will lead to the formation of a large, coarse ice structure. And the faster rate will be favored to the formation of small, dendritic ice structure. But the influence on the real formation is to be further explored for better quantification.

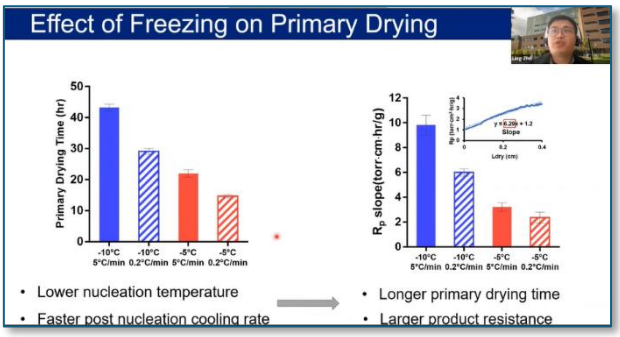


With that introduction, I would like to address the first part of my research, what is the effect of freezing on primary drying?

In order to solve these questions, in our research we use 5% weight by volume sucrose solution as a model compound. And the sucrose solution was prepared in a pre-cut 2cc vials, as shown in this figure. And the filling depth of the solution is seven millimeters. Besides that, we also design for freezing protocols from fastest to slow. The protocol was shown in the following tables. And the protocol with the fastest rate is achieved by setting the ice nucleation temperature at -10 degrees C with the post shelf con rate at five degrees per minute. The next slow rate is achieved by changing the shelf con rate from five degrees per minute to 0.2 degrees per minute by keeping the ice nucleation temperature constant. The slowest rate was achieved with a higher ice temperature at minus five degrees and a post shelf cooling rate at 0.2 degrees per minute. In experiment, the minus five degrees ice nucleation temperature is hard to achieve, so we use annealing as a replacement.



Since in our research we want to test the exclusive influence of the freezing, the product temperature has to be controlled during the primary dryring. From the last column of the table we can see that different shelf temperature was applied and then increase of shelf temperature as high as 10 degrees C can be applied to achieve the same product temperature as shown the second and last column. And during our research for all the freezing cycles, the secondary drying conditions were same and all the freezing conditions were established based on the freeze dryer provided by the Millrock Technology.

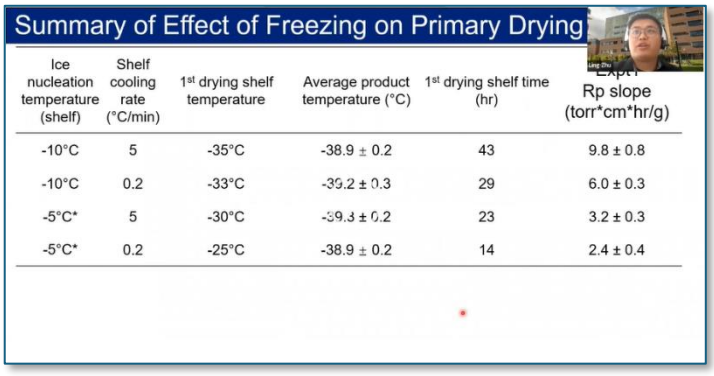


From that we first see how the primary drying is influenced by the freezing conditions. The primary drying time for each cycle is shown in the left figure in this slide. And in this figure the blue bars show the result for the nucleation temperature at -10 degrees C while the red bars is the result for the minus five degrees C ice nucleation temperature. The solid bars show the result for the post-nucleation cooling rate at five degrees C per minute while the dashed bar are the result for 0.2 degrees C per minute.

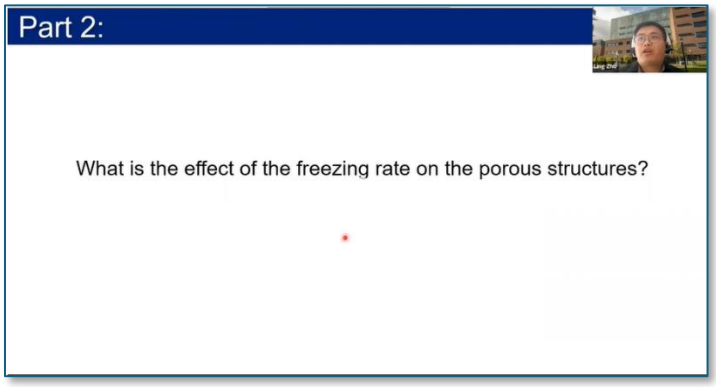
If we look into the effect of the ice nucleation temperature, we can find that by keeping the post-nucleation cooling rate constant, the cycle nucleated at -10 degrees C will give us a longer primary drying. If we look into the effect of the post-nucleation cooling rate, the cycle with five degrees per minute will give us a longer primary drying. Besides that, we also looked into the product resistance that changed with the freezing conditions. As shown in the right figure, the product resistance slope in this figure was used to evaluate how difficult it is for water molecules to escape the vials during primary drying. And during the primary drying, we used the single vial data to calculate the product resistance and describe the change of the product resistance versus trial and error thickness.

By plotting the product resistance with trial and error thickness, we can get the product resistance slope. For people that are familiar with the equation shown here, the product resistance slope is the A1 in this equation. The product resistance slope for the freezing conditions shows the same trend with the primary drying time and from both figures we can see that a lower nucleation temperature and a faster post-nucleation cooling rate will give us a longer primary drying time with larger product resistance.

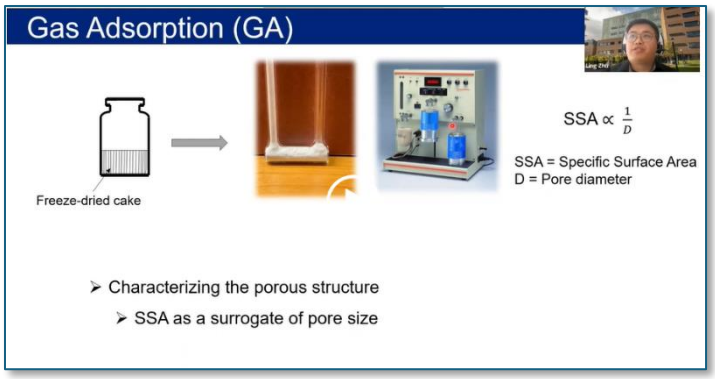
The result of the primary drying time and the product resistance slope is summarized in the following table, we can see that with appropriate freezing protocol you will be able to increase the shelf temperature during primary drying to increase the sublimation rate without compromising the product temperature and the quality. By doing this, the primary dry time can be reduced by two out of third with the product temperature remaining the same.

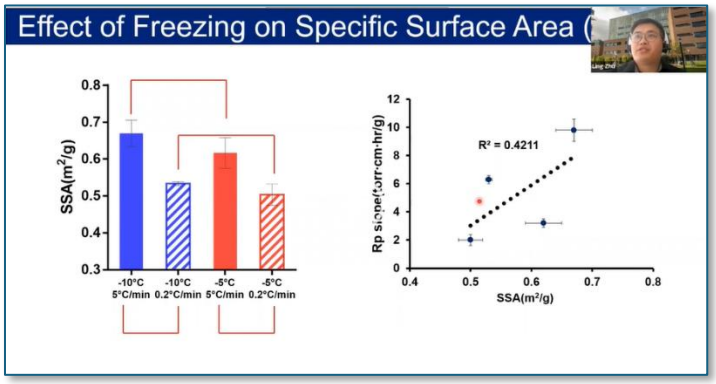


After understanding the freezing effect on the primary drying, the second part of my research is focused on what is the effect of the freezing rate on the porous structures? And in order to address that, it is important to characterize the porous structures.



After understanding the freezing effect on the primary drying, the second part of my research is focused on what is the effect of the freezing rate on the porous structures? And in order to address that, it is important to characterize the porous structures. And traditionally in the pharmaceutics areas, to characterize the lyophilized cake, we used the gas absorption as the golden standard method. As shown in the following pictures, during the merriment, the lyophilized cake in the vial was disrupted and transferred to a special container in this figure. And this container will later connect to the machine to measure the general specific surface area of lyophilized cake. And by this method, it will give us the general specific surface area which is labeled as SSA here. In this method the specific surface area was used as a surrogate of the pore size. . The specific surface area has an inverse relationship with the pore diameters,



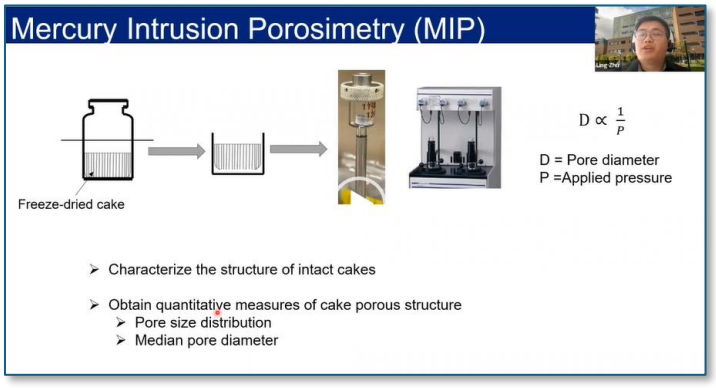


which means that the larger the specific surface area was measured, the smaller the pore diameter it will be.

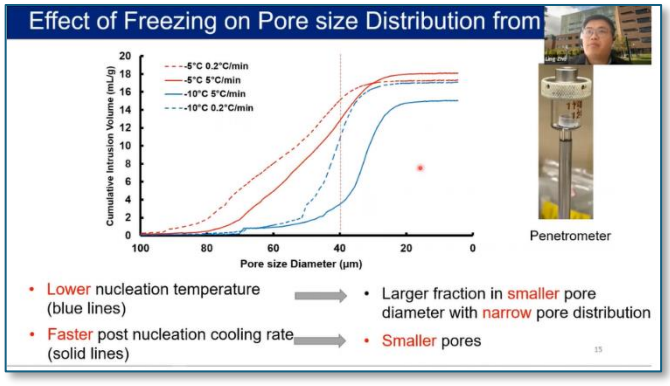
From that. Let's move to the result of the gas as option and see how the freezing influenced on the specific surface area. As shown in the left figures, if we look at the effect of changing the post-nucleation rate and look at the blue bars in this figure we can find that when changing the faster freezing rate from five degrees C per minute to a slow rate at 0.2 degrees C per minute, the specific surface area decreased. A similar result was found for the red bars. It indicated that a faster post-nucleation cooling rate will cause the increase of the specific surface area. However, when we look into the effect of ice nucleation temperature on the specific surface area only a slight difference can be observed between the group of -10 degrees C and the nucleation temperature at minus five degrees C. And from left to the right on the X axis the rate changes from the fastest to slowest. No obvious trend can be found between the specific surface area and the freezing rate.

When we further correlate to the specific surface area and the product resistance slope, which is shown in the right figure, we find the point has a scattering distribution. In other words, the product resistance slope vaguely correlates with the specific surface area. However, we know there is a better correlation found in Ramda's paper in 2004, so it could be in our cases, this method has some limitations. And since the specific surface area cannot especially capture the features of the lyophilized cakes, we turn to the other methods to continue the characterization.

And the second method in our research used is the mercury intrusion porosimetry, which is labeled as an MIP here. In this method, the freeze-dried cake is carefully capped in a precut while and transferred intact shape to the penetrometer, shown in this figure. The penetrometer will later be used for equipment for test. During the measurement, a series of pressure was applied to push the mercury through the penetrometer stamp and to penetrate into the pores in the lyophilized cakes. In the measurement, the measured pore diameters have an inverse relationship with the applied pressure. And at each pressure we got one pore diameters. And when combine all the pore size, we can get the pore size distribution. And besides, we can also get the median pore diameters with the lyophilized cake.



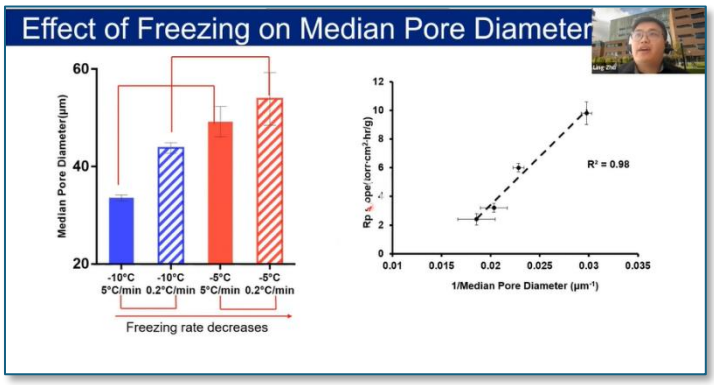
The result of the pore size from the mercury intrusion porosimetry is shown in the following figure. The Y axis shows the cumulative intrusion volume



of the mercury that penetrated into the lyophilized cake during the measurement. And the X axis is in the inverse order of the pore size. This is because the pore size is inverse to the pressure and the pressure continues to increase during the measurement. In this plot, every increments of the intrusion volume of mercury at a specific pore size means the amount of the mercury intruded into the pore and the larger increase of the intrusion volume or the steeper of the curve showing the figure at a specific pore size indicated that more fraction of this pore can be found in the cake. And shown in the plot. The blue lines is the result at -10 degrees C ice nucleation temperature, and the red lines are the result for minus five degrees C. And the solid line is the result for five degrees C per minute post the nucleation cooling rate and the dashed line is result for the 0.2 degree C per minute.

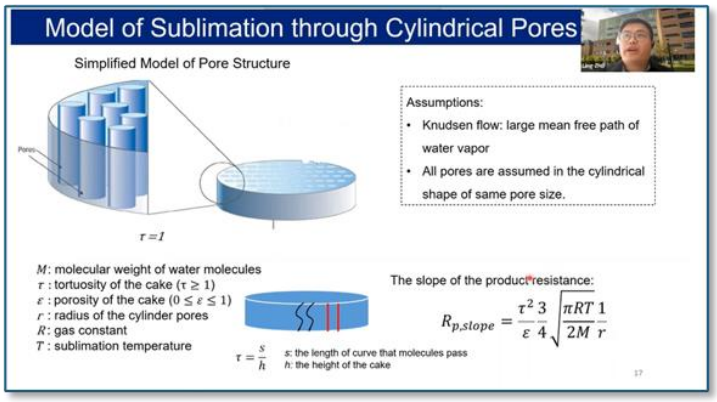
And from the figure there are three highlights. If we use the 40 microns as a benchmark, we can find that the blue lines have more pores in the right part of the 40 microns. Compared to that, the red lines have more pores on the left part to the 40 microns. This means that under the -10 degrees conditions, they will have smaller pores. Also, when we look into the pore size distribution of each line, we find that the blue lines show a narrow pore distribution, and all the pores are in the range between 30 microns to 50 microns. While the dotted lines show the wider pore distribution with all the pores ranging from 30 microns to 90 microns.

Based on these two findings, it indicated that a lower ice nucleation temperature will result in more small pore size and a narrow pore size distribution in lyophilized cake. Besides, we can also find that when we compare the solid lines result with the dashed line result, all the solid lines are on the right to the dashed line towards the smaller size area. That means the five degrees C per minute condition has more pores forming the small pores areas compared to the 0.2 degrees C per minute conditions.



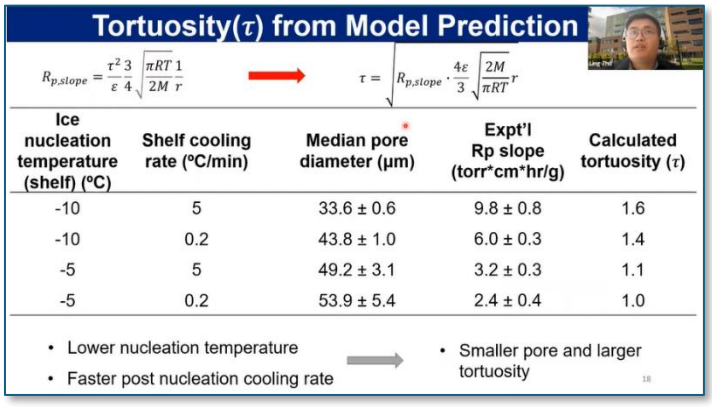
And from the pore size distribution above, we can also find that the median pore diameters for the lyophilized cake from each freezing particles. The median pore diameters is measured based on the mercury intrusion volume. And from the figure on the left we can find that as the freezing rate increases from the left to the right, the median pore size increases. When looking at the effect of the ice nucleation temperature and the post-nucleation cooling rate, a higher ice nucleation temperature and a slower post-nucleation cooling rate will lead to a large median pore size.

When further correlated with the product resistant slope, we found that all the points follow a line and there's a strong correlation between the reciprocal of a median pore diameter and the product resistance slope. It indicated that the result from the mercury intrusion porosimetry symmetry shows some pore features that could influence the product resistance. And to further understand this correlation, we used the simplified model.



As shown in this figure, we simplified the cake with many pores and all the pores in this cake have same shapes. In order to better understand this model, I would like to introduce the concept of tortuosity. As shown in this figure, tortuosity is the term used to describe the ratio of the paths that water molecules have to escape the vial, shown in these black lines, to the height of the cake, which is shown in the red lines. And when the path equals the height of the cake, as shown in this top figure, the tortuosity equals one.

And to use this model we made two important assumptions. First, the water will follow the Knudsen flow. In this flow, nucleation happened between water molecules. Second, all the pores in the lyophilized cake are assumed to have the same cylindrical shape with the same pore size. And from that, the product's resistance can be derived, shown as the following equations. And in this equation, we find that the product resistance slope, sorry, is inverse to the radius of the pore size. This could explain the result we found from the previous slides that the product resistance slope has the good correlation with the reciprocal of the measured median pore diameter. And the larger the pore radius is, the smaller the product resistance will be and make it easier for the ice to sublimate. Also in this equation, we can also find other variables, porosity and tortuosity. And these two factors could also influence the product resistance slope.

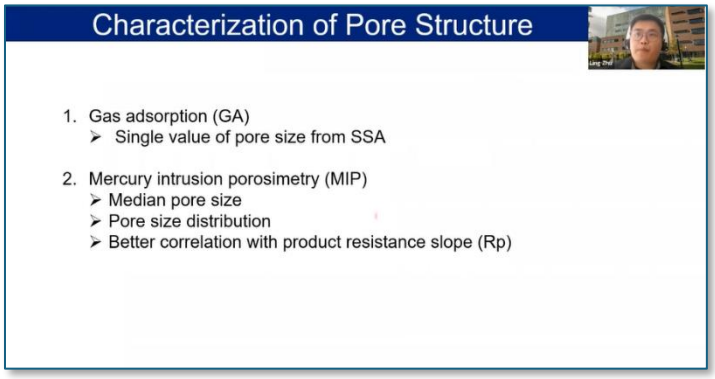


We are interested in how these changes of variables influenced the product resistance. So, in our experiment the product temperature was controlled the same during primary drying and it is kept at a low temperature for all the cycles. So, for the lyophilized cake, no cracks and no shrinkage were expected and observed in the final lyophilized cake with the same formulation. In this case the porosity should be constant for all the four cycles. This is also verified by the mercury intrusion porosimetry measurement. And in this case from this equation, what we are interested in is the tortuosity changed by the freedom.

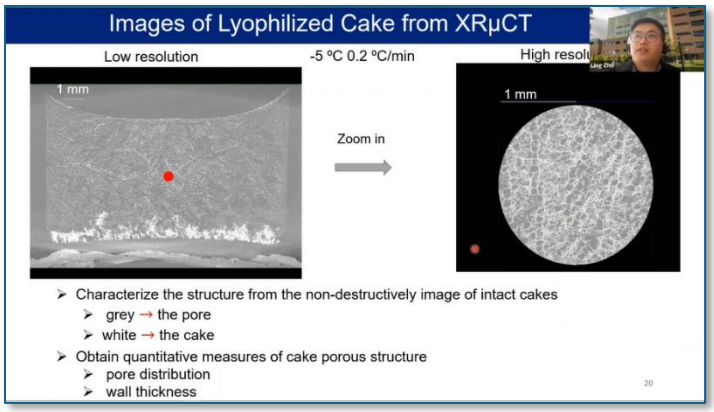
If we use this model and then tortuosity can be determined in the following equation, and if we use the data from the merriment of mercury intrusion porosimetry, we can get the description on the tortuosity of the cake for each cycle, which is shown in the last column of the table.

From the table we can find that when the freezing rate changes from slow to fast, the median pore diameters decreases and the product resistance slope increases. We can also find that the tortuosity of all the lyophilized cake increases. This makes sense because the small pores may have more pores. And from the table we can see that the lower nucleation temperature and the faster post-nucleation cooling rate will lead to the formation of smaller pores and the later tortuosity in the final lyophilized cake.

From that, what we have learned so far about the characterization of pore structure is shown in this slide. We learned that the gas absorption gives us the single value of the pore size from the general specific surface area. We also learned that the mercury intrusion porosimetry provides more information about the porous structures in the lyophilized cake, such as the median pore size and the pore size distribution. And a better correlation between the product resistance and the median pore size is observed.

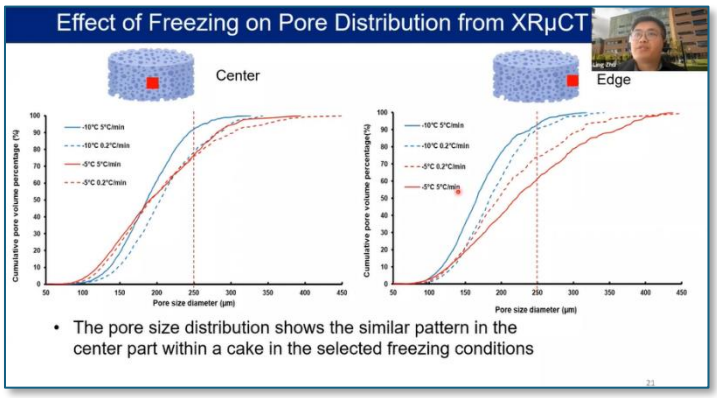


However, one point you need to mention about the two methods is that in both methods, worth barely knowing is the geometry of the pores and the pore distribution in the cake. In this case, we export the third method in the research that can give us more detailed information about pore features. And the third method we use in our research is X-ray micro-CT imaging. In this technique, the lyophilized cake is transferred to a plastic container and carefully sealed in the container. During the measurement, the whole lyophilized cake rotated with the X-ray exposure and the result is shown in the following pictures.



On the left, it shows the result of lyophilized cake in a vertical cross section around the middle of the cake. This image is from the non-destructive image of intact cake. In the image, the gray part is the pores, and the white part is the solid cakes. The white part at the bottom is the glue to stabilize the cake during the scanning. And this image is what we got from one of the lyophilized cakes produced under the condition of minus five degrees C and 0.2 degrees C per minute.

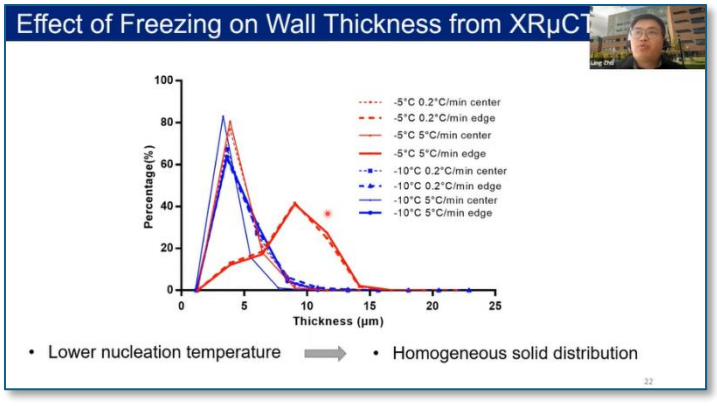
When we look into the image, we can see that there are several different shapes of the pores in this cake. Typically, if we look at the center parts, we can see that most pores are in a tiny star shape. If we move to the edge area of the cake, we can see some elongated shape of the pores. Some of them are along the directions from the bottom to the top, some are in the horizontal directions from the edge to the center in a feather like pattern. And if we pick one point from the image and zoom in, we can see more details.



As shown in these right figures, if we pull some point at the half of the sickness in the center of the cake and zoom in the horizontal plane of the XY section, we can find that the pores in this plane are in a dot shape with variety in the pore sizes. And the white lines showing this picture represent the wall thickness that indicate the solid cake distribution. A thick wall thickness usually combined with a small pore size. The quantitative result is analysis with imaging software by using a high resolution result image. For each freezing conditions we pick one lyophilized cake for imaging analysis. And in each cake, we pick one point in the center and one point in the edge to show the pore distribution.

And the result will be shown in the following pictures. The left figure is a result of the distribution in the center of the cake. And this figure shows the percentage of cumulative pore size distribution in the selected area as the function of the pore size diameter. And every increment on the curve at the specific pore diameter shows the volume percentage of the pore at that diameter. From the result, we can see that for all the freezing conditions in this research, only slight difference can be observed in the center part for the pore distribution. And the pore distribution shows us similar patterns in a certain part with cake in the selected freezing conditions in our research. That indicates that the freezing conditions might not influence the pore distribution in the center part of the cake.

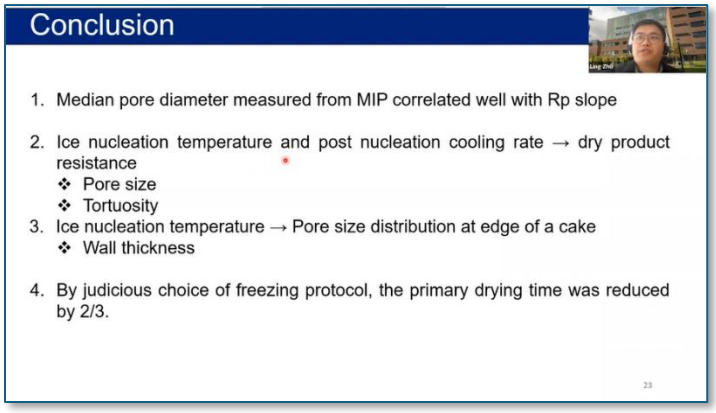
While in the right figures, when we look into each part of the cake, we can find that the pore distribution has different patterns. The right two lines show the results of the ice nucleation temperature at minus five degrees C. And under this condition, they have larger pores compared to the result from the two blue lines. And if we use the 250 microns as a benchmark in both figures, we could find that for the blue lines, the pore distribution are the same in both the center part and the edge part. However, for the red lines, the pore distribution in the edge part is different from the center part and the



larger pores can be found in the edge areas. This indicates that by using a higher nucleation temperature, the larger pores can form in each area within a cake.

Besides, we also look into the pattern of the wall thickness in the lyophilized cake. As shown in this figure, the Y axis means the percentage of a specific wall thickness in the selected part of the cake and the X axis is the thickness. In this figure, the blue line shows the result of the ice nucleation temperature at the -10 and the red line is the result for minus five. The solid line is five degrees C per minute for the post-nucleation cooling rate and the dashed line is 0.2 degrees C per minute. The thick line in this figure is the result from the edge and the [inaudible 00:33:45] is the center. What we can find in this figure is that all the blue lines have a similar wall thickness distribution, which indicates that at the -10 degrees C conditions, the wall thickness or the solid distribution is not dependent on the point selected in a cake. And it indicates that the lower nucleation temperature will tend to give us a more homogeneous solid distribution with cake.

We also find that the red lines have two separate results. The center part has the same wall thickness distribution as the blue lines in the small wall thickness areas. However, the pore distribution in the edge is different. We could say that more solid in the edge has larger wall thickness. This correlates well with the pore distribution in the previous slide, and it indicates that the higher nucleation temperature will tend to form lyophilized cake with larger wall thickness formed in the edge part.



With that, I'd like to give a general conclusion for today's webinar. First, we learned that the median pore diameter measured from the mercury intrusion porosimetry correlates well with the product resistance slope. And second, from the mercury intrusion porosimetry result, we find that both the ice nucleation temperature and the post-nucleation cooling rate will influence the dry product resistance by changing the process and the tortuosity. And when the ice CLE temperature decreases and the post-nucleation cooling rate increases, the product resistance rate increases, which turn out to have smaller pores in the lyophilized cake, with a larger tortuosity. And from the result of x-ray micro-CT imaging, we can see that the ice nucleation

temperature influenced the pore distribution and the wall thickness within a cake in different parts. And the major difference was on the edge area of the cake.

And last, from the effect of the freezing on the primary drying, we can see by judicious choice of freezing protocol, the primary drying time was reduced by two out of the third.

With that, I'd like to thank the help and support from my advisor, Dr. Robin Bogner, for her kind help on this project. And thanks to my lab members, Moshina and Dinesh, and our collaborators, Dr. Sina and Yara. And thanks for the funding support for CPPR and the equipment supplied by Millrock Technology. With that, I am open to questions.