CRYODRY®

Theory to Practice

CryoDry® Freeze Drying Best Practice and Techniques

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What is Freeze-Drying?

Freeze-drying (also known as lyophilization) is a process of removing water or other solvents from a material through sublimation. Sublimation is the direct transition of water from the solid (ice) phase, to the gaseous (water vapor) phase, without passing through the liquid phase. This process preserves the structure and physical properties of the product.

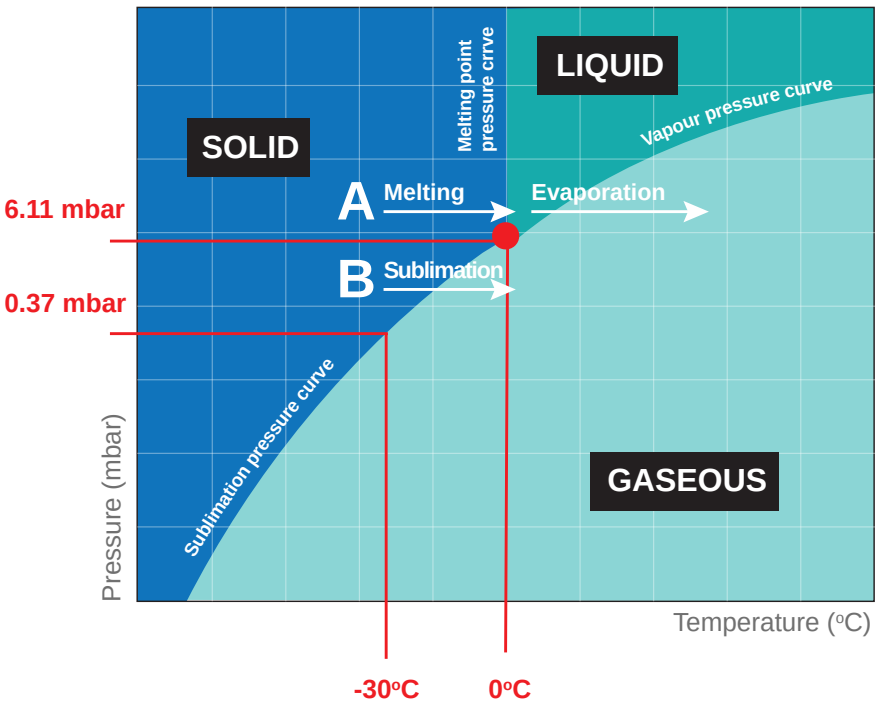
What Applications can Freeze - Drying be Used for?

Freeze-drying has a wide range of applications including the preservation and production of food and pharmaceutical products, biological materials, and other perishable products. In the food industry, freeze-drying is used to produce things such as instant coffee as well as to preserve and extend the shelf life of various types of food such as fruits and vegetables. In the pharmaceutical industry, freeze-drying is used to produce stable and long-lasting vaccines, antibiotics, and other medical products. In the cannabis space freeze drying is used to dry flower and bubble hash. Biological materials (such as cells, tissues, and enzymes) are also commonly freeze-dried for storage and transportation purposes. Other applications of freeze-drying include the preparation of certain types of polymers and ceramics, and the preservation of rare or fragile documents and artworks.

How does the Freeze Drying Process Work?

If an aqueous product is frozen, and energy (in form of gentle heat) is applied at a pressure below the triple point of water, the product will undergo sublimation: directly transforming from a solid to a gaseous state without passing through the liquid phase. Moreover, the vacuum element of freeze-drying can prevent the melting of ice when energy is added. This is significant for freeze-drying as, when thermal energy is added to a frozen product under vacuum, thawing of the product is prevented. Instead, the water that is contained within the product is released in the form of water vapor, thereby preserving the quality of the product.

As an example, if thermal energy is added to pure ice with a temperature below –30°C and a pressure of 0.37 mbar, it is converted into water vapor once it reaches –30°C.



Freeze-Drying System Design

The main components of a standard freeze-dryer include:

- A vacuum drying chamber or manifold
- A vacuum pump for generating vacuum inside the drying chamber
- An ice condenser to act as cryo-vacuum-pump and for collecting the water vapour that is released by the product

The CryoDry® CD8 freeze-dryer has an efficient design that includes a combined drying chamber and condenser. For most applications this design is preferred over separate drying and condenser chambers, due to the short and unrestricted vapour flow path which makes it far more efficient.

What are the freeze-drying process steps?

From a physical standpoint, the freeze-drying process can be divided into three distinct phases:

(1) **Freezing:** The product to be dried is frozen at atmospheric pressure, either directly in the freeze-dryer, or in a separate freezing tool such as deep-freezer, or by exposure to dry ice or liquid nitrogen. The freezing temperature should be approximately 10°C below the solidification point of the product, however in most cases other than controlled-rate-freezers and certain types of freeze dryers, users do not actually have the ability to control the freezing temperature. This inability to control the first (yet very important) process step can lead to a number of process issues.

(2) **Evacuation:** Once the product is sufficiently frozen the vacuum pump is activated, and the pressure inside the drying chamber is lowered to the corresponding value that aligns with the freezing temperature following the vapour pressure curve for ice and water. Basic freeze dryers do not have the ability to precisely control pressure which can lead to negative melt-back effects if too high or can cause excessive drying times if too low.

(3) **Sublimation:** Thermal energy is added to the product, initiating the sublimation process. The water in the product is transformed into water vapour due to the added energy. As the ice condenser is colder than the product, the vapour pressure in the ice condenser is significantly lower than that above the product. Consequently, the water vapour released by the product moves to the ice condenser, where it condenses and re-freezes on the condenser coils.

The sublimation phase is separated into main drying and secondary or final drying phase.

During the main drying phase free water is extracted from the product. The final traces of bound water will be removed in the secondary drying phase at a final pressure that is as low as possible and at higher temperatures. This is accomplished through a process called desorption.

Freezing

The CryoDry® CD8 "All-in-One" process allows direct freezing of the product inside the chamber, eliminating the need for external freezing technology and eliminating necessary labour for sample transfer from freezing solution to dryer.

- Products freeze either as crystalline and amorphous structures, with most freeze-dried products having a crystalline form.
- Crystal size impacts on drying time and resulting product quality. To avoid smaller ice crystals that can negatively impact the drying process, quick and deep freezing should be avoided. Higher freezing temperatures and slow temperature reduction are preferable.
- The solidification point of the product needs to be determined to ensure an optimal freeze-drying process, with the product temperature generally kept 10°C below this point.

Primary Drying

After the product is frozen, the main drying phase begins, and the vacuum pump is activated. The pressure inside the drying chamber is lowered to correspond with the sublimation pressure curve for ice and water, which is the value that corresponds to the freezing temperature.

Thermal energy is then added to the product. This is done in 3 ways:

1 Conduction from the heated shelves into the product
2 Radiation from heated shelves above the product
3 Convection currents in the chamber

This initiates the sublimation process. The maximum drying rate is achieved at the start of the process. As the sublimation area recedes into the product, the resulting water vapor must pass through the already-dried layers. This is where large crystals with bigger pores allow better vapour transport thus creating faster and more efficient drying conditions.

The drying time depends heavily on the drying vacuum. For instance, at 1.0 mbar, one gram of ice takes up a volume of 1 m^3 of vapor; while at 0.1 mbar, it takes up a volume of 10 m^3 of vapor; and at 0.001 mbar, it takes up a volume of 100 m^3 of vapor. The closer the vacuum is to the solidification point, the smaller the resulting vapor volume, which leads to an increase in the drying rate and a decrease in the drying time.

Secondary Drying

Secondary drying is the process of removing any remaining moisture from the product after primary drying. This is done by increasing the shelf temperature to drive off the remaining moisture. The process is typically carried out at a higher temperature and lower vacuum level than primary drying.

The duration of the secondary drying phase depends on the properties of the product being dried, such as its composition, moisture content, and the intended shelf life. The temperature and pressure conditions during secondary drying are optimized to ensure complete removal of the remaining water molecules, while avoiding

degradation of the product. The result is a dry, stable product that can be stored at room temperature for long periods of time.

End of Drying

Once secondary drying is complete, the vacuum pump can be turned off, and the chamber vented to bring it back to atmospheric pressure. The product can be removed after opening the door. However, it is crucial to store the product correctly as it can be highly hygroscopic following freeze-drying making it prone to moisture uptake.

Defrosting

After each run the unit should be defrosted.

How to Determine a Freeze-Drying Recipe?

When first setting up a freeze-drying recipe there are some critical data points that need to be established. These include:

1. **Freezing Point of the Product:**
   If you are freeze-drying an aqueous product you might expect that the freezing point is 0°C. However, in practise this would not likely be the case. Firstly, the addition of product into the water will affect the freezing point (lower). Secondly you will often see a "super-cooling" event. This is where the temperature of the product will drop well below the freezing point as much as -10 or -20°C. Eventually a nucleation event takes place, which is when the product freezes. This nucleation event is exothermic (gives off heat) and can be detected by a rise in the product temperature. After this nucleation event the product should be frozen.

2. **Critical Temperatures:**
   Once the product is frozen you need to input energy in the form of heat to drive the sublimation process. If you input too much heat, the product will melt which means the process is no longer freeze drying, but evaporating. Note that melting is generally not a direct effect of the energy input into the product, but is because the resulting vapour generated exceeds the condensing and pumping capacity of the ice condenser and vacuum pump which leads to a pressure rise, and subsequent product temperature increase. To ensure this doesn't happen the **collapse temperature** should be determined. The collapse temperature determines the maximum temperature that a product can reach in primary drying without melting or collapsing. This is usually determined by either Differential Scanning Calorimetry (DCS) or Freeze-Drying Microscopy (FDM). Once you have determined the collapse temperature you would set a target temperature 2-5°C below the collapse temperature.

3. **Vacuum Setting:** A common misconception is that a lower vacuum is better because it "sucks" the water out of the product faster. This is not the case, rather, the primary driver for sublimation is the amount of energy input into the product. Too low a vacuum can slow down the sublimation process, because it generates excessive amounts of vapour volume. A good starting point is to take the collapse temperature and determine the Vapour Pressure of Ice using our table on the next page.

Vapor Pressure of Ice

In contact with its own vapor

Temp	Vapor Pressure			Temp	Vapor Pressure		
°C	Pa	mbar	Torr	°C	Pa	mbar	Torr
0	611.0	6.110	4.583	-42	10.22	0.1022	0.0767
-2	517.7	5.177	3.883	-44	8.10	0.0810	0.0608
-4	437.4	4.374	3.281	-46	6.39	0.0639	0.0479
-6	368.7	3.687	2.765	-48	5.03	0.0503	0.0377
-8	309.9	3.099	2.324	-50	3.94	0.0394	0.0296
-10	259.9	2.599	1.949	-52	3.07	0.0307	0.0230
-12	217.3	2.173	1.630	-54	2.38	0.0238	0.0179
-14	181.2	1.812	1.359	-56	1.84	0.0184	0.0138
-16	150.6	1.506	1.130	-58	1.41	0.0141	0.0106
-18	124.9	1.249	0.937	-60	1.08	0.0108	0.0081
-20	103.0	1.030	0.773	-62	0.82	0.0082	0.0062
-22	85.07	0.851	0.638	-64	0.62	0.0062	0.0047
-24	69.88	0.699	0.524	-66	0.47	0.0047	0.0035
-26	57.23	0.572	0.429	-68	0.35	0.0035	0.0026
-28	46.71	0.467	0.350	-70	0.26	0.0026	0.0020
-30	38.00	0.380	0.285	-72	0.19	0.0019	0.0014
-32	30.81	0.308	0.231	-74	0.14	0.0014	0.0011
-34	24.89	0.249	0.187	-76	1.10	0.0011	0.0008
-36	20.03	0.200	0.150	-78	0.08	0.0008	0.0006
-38	16.07	0.161	0.121	-80	0.05	0.0005	0.0004
-40	12.84	0.128	0.096	-82	0.04	0.0004	0.0003

1 mbar = 750.1 microns
1 mbar = 100 Pa

1 micron = 0.1333 Pa
1 micron = 0.0013 mbar

1 Pa = 7.5006 microns
1 Pa = 0.01 mbar

1 micron = 0.1333 Pa
mbar (cgs units) = milibar (10^3 dyns/cm sq)
microns = micrometers of mercury
Pa (SI units) = Pascal (N/m^2)
micron = µmHg = mTorr

4. **Shelf Temperature:**
After the initial freezing of the product has been achieved the shelf temperature needs to be increased and if possible the product temperature should be monitored at the same time. You will find that, the product temperature will be much lower than the shelf temperature due to the effect of evaporative cooling. The shelf temperature is increased in a number of steps until the product temperature reaches the target temperature. Anything higher may result in the melting or collapse of the product.

5. **End of Primary Drying:**
The end of primary drying is determined by the shelf temperature being the same as the product temperature. Note that not all freeze dryers can measure product temperature. Our CD 8 does not currently have this feature, but you could use an external temperature logger to gain these insights. It is important to use thin temperature sensor wires. We recommend using a sealing agent such vacuum grease or silicone to reduce the generation of vacuum leaks.

6. **Secondary Drying:**
If a very low residual moisture level is required a **secondary drying** step can be added. At this point it is safe to increase the shelf temperature above the critical temperature as all of the free water (that is, ice) has been removed. **Secondary drying** vacuum setting is often much lower than **primary drying**.

7. **Practical Example**
If the product has a collapse temperature of -30°C:

   a. The target temperature is set at -33°C (2-5°C below the collapse temperature)
   b. The Vapour Pressure of Ice for -30°C is 0.38 mbar
   c. Shelf temperature is set and the product temperature is monitored

      i. Shelf Temp -30°C Product Temp -38°C
      ii. Shelf Temp -20°C Product Temp -33°C*

*The product temperature has reached the target temperature, so the shelf temperature should be maintained at -20°C until primary drying is completed.

Step-by-Step Recipe Development:

Step 1:
Measure the freezing point of your product if you can or try to determine via literature. If you do not know it, then use our CD8 freeze temperature control function and work in small steps from 0°C downwards e.g. 0°C, -5°C, -10°C, -15°C, and so on in order to determine it. Remove the product from the dryer after allowing sufficient time to freeze (more time is safer). Use small amounts of product for shorter freezing time. Note that the time invested to determine your product freezing point will pay significant yields over future runs by giving you shorter drying times and better quality product.

Step 2:
Determine your lowest permitted pressure, (i.e. ice temperature, which must remain >10°C higher than the ice condenser temperature to retain cryo-pumping effect). For our CD8, this is typically 0.37 mbar (or -30°C). This gives you the best process speed during the main drying phase.

Step 3:
Determine a safe ice temperature that prevents melting of ice, but is as high as possible (in order to keep the produced water vapour volume at minimum). This is typically >5°C below product melting temperature.

Step 4:
Program a ramp in a few steps with the following principle:

1) **Product Freeze:**

- No vacuum.
- Reduce shelf temperature gradually to >10°C below product freezing point.
- Allow sufficient time for product to fully freeze at the set freezing temperature. The more product in a batch, the more time is required. The thicker the product (i.e. the ice layer) the longer it takes. Manually check progress by temporarily removing the product.
- It is common for freezing to take 6-8 hours.

2) **Primary Drying**

- Apply vacuum in line with above.
- Set shelf temperatures in line with above.
- Increase shelf temperature gently in a few steps.
- You can reduce pressure, but often one or few steps are is sufficient.
- Use relatively long periods for each step.
- It is common for primary drying to take 8-12 hours.

3) **Secondary Drying**

- Generally only one step is sufficient required, but you may choose to program more.
- Increase shelf temperature to the maximum temperature that is safe for your product.
- Reduce vacuum significantly, (this can be as low as ultimate vacuum of the system).
- It is common for secondary drying to take 2-4 hours.

Common Freeze-Drying Recipe Parameter Framework:

Disclaimer: Below parameter sets and example recipes are recommendations only, and may not provide best possible drying times and product quality for your specific product or sample. Freeze-drying results depend just as much on the product itself as they do on the correct recipe. We recommend adjustment to your specific needs.

Fruit General (Chopped 1-2 cm)

Framework:
Freezing Temperature <-35°C or max cold, 4-8h.
Main Drying Pressure 0.63 to 0.22 mbar.
Final Drying Pressure 0.12 to max vacuum.
Main Shelf Temperatures -30 to 10°C in 5-10°C steps.
Final Drying Shelf Temperature 20°C depending on temperature tolerability.
Drying Section Times 4-8h.
Total Process Time 24 to 48h.

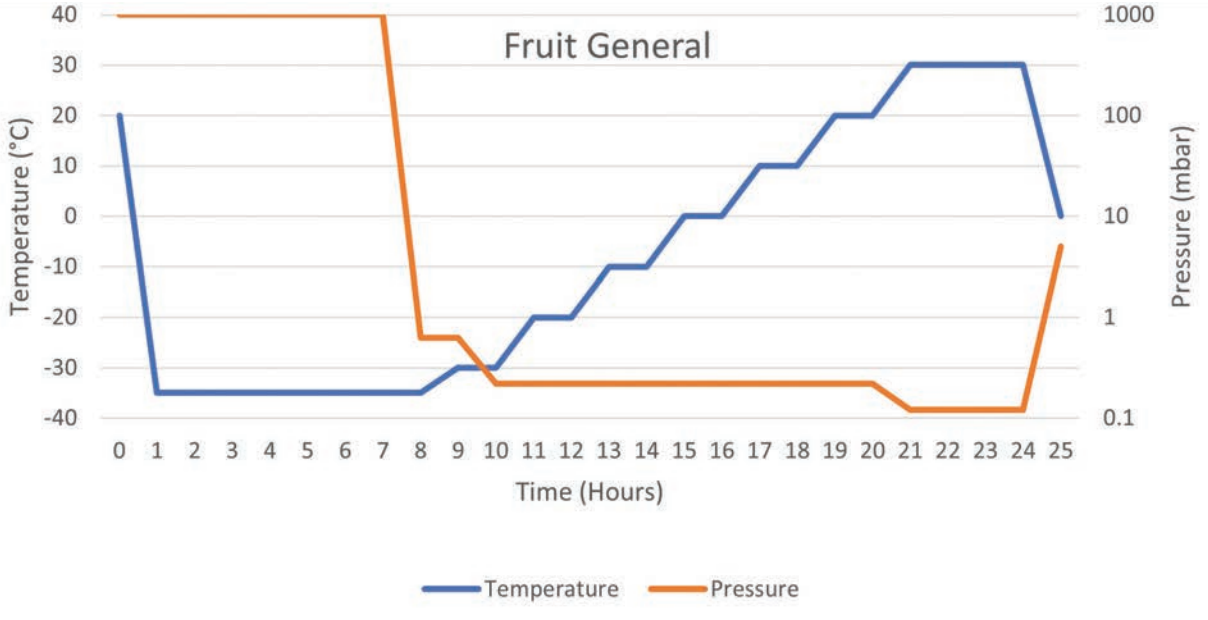
Example Parameter Set:

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-35
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	40

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-30	120	0.63
2	-20	120	0.63
3	-10	120	0.37
4	0	120	0.37
5	10	120	0.22
6	20	120	0.22
7	30	240	0.12



Vegetables (Chopped 1-2 cm)

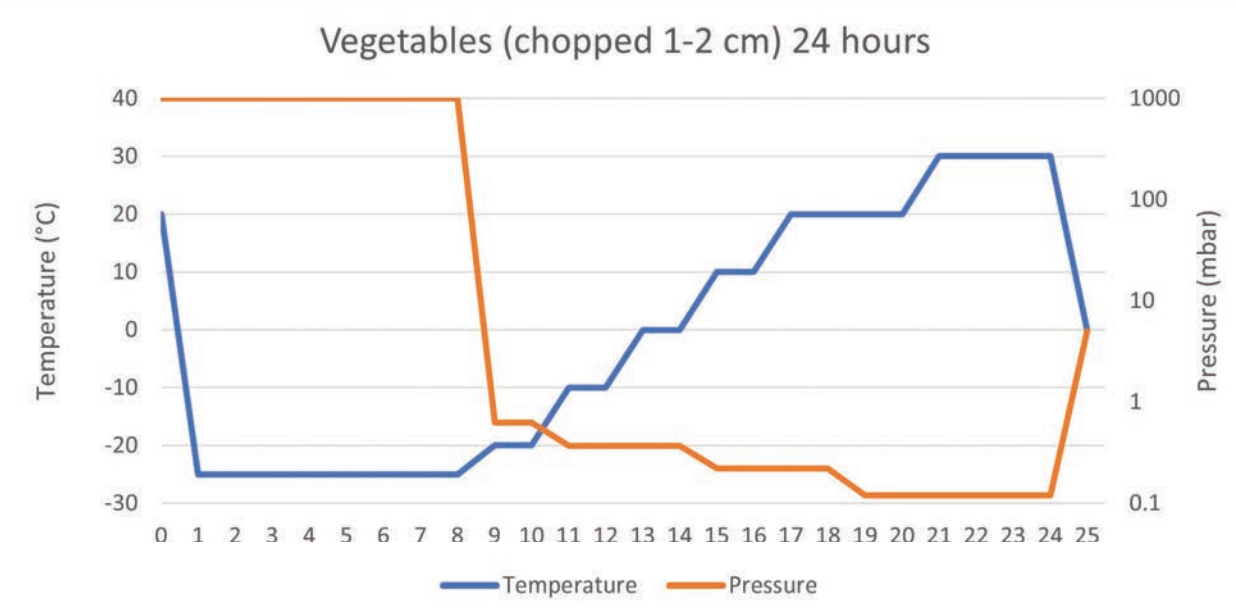
Freezing Temperature <-20°C or -30°C, 4-8h.
Main Drying Pressure 1.65 to 0.37 mbar.
Final Drying Pressure 0.22 to 0.12 mbar.
Main Shelf Temperatures -20 to 10°C in 5°C steps.
Final Drying Shelf Temperature 10-30°C depending on temperature tolerability.
Drying Section Times >4h.
Total Process Time 24 to 48h.

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-30
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	40

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	120	0.63
2	-10	120	0.37
3	0	120	0.37
4	10	120	0.22
5	20	240	0.22
6	30	240	0.12



Cannabis Flower

Freezing Temperature <-25°C, 6-8h (only needed if product is fresh or not externally frozen).
Main Drying Pressure 1.65 to 0.63 mbar.
Final Drying Pressure 0.63 to 0.12 mbar.
Main Shelf Temperatures -20 to 10°C in 5-10°C steps.
Final Drying Shelf Temperature 20°C max.
Drying Section Times 2-4h.
Total Process Time 24-36h if freezing included, 18-24h if product is frozen.

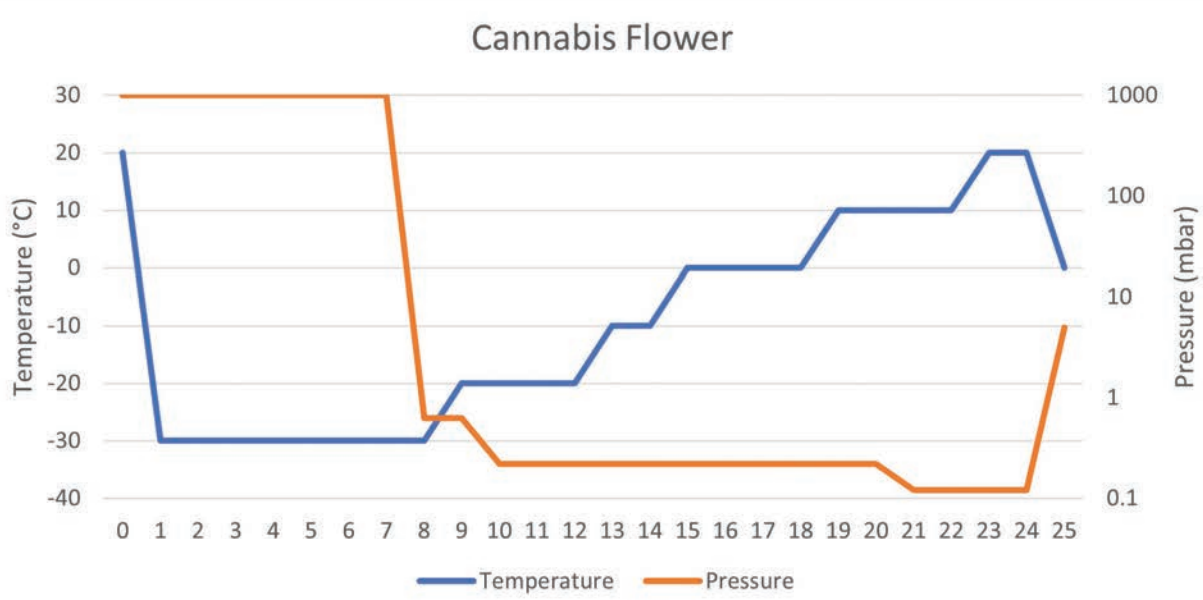
Example Parameter Set:

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-30
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	35

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	120	1.65
2	-20	120	1.03
3	-10	120	0.63
4	0	240	0.63
5	10	240	0.22
6	20	120	0.12



Bubble Hash

Freezing Temperature <-25°C, 6h (only needed if product is fresh or not externally frozen).
Main Drying Pressure 1.65 to 1.03 mbar.
Final Drying Pressure 1.03 to 0.63 mbar.
Main Shelf Temperatures -20 to -5°C in 5°C steps.
Final Drying Shelf Temperature 0°C max.
Drying Section Times 2-4h.
Total Process Time 24-36h if freezing included, 18-24h if product is frozen.

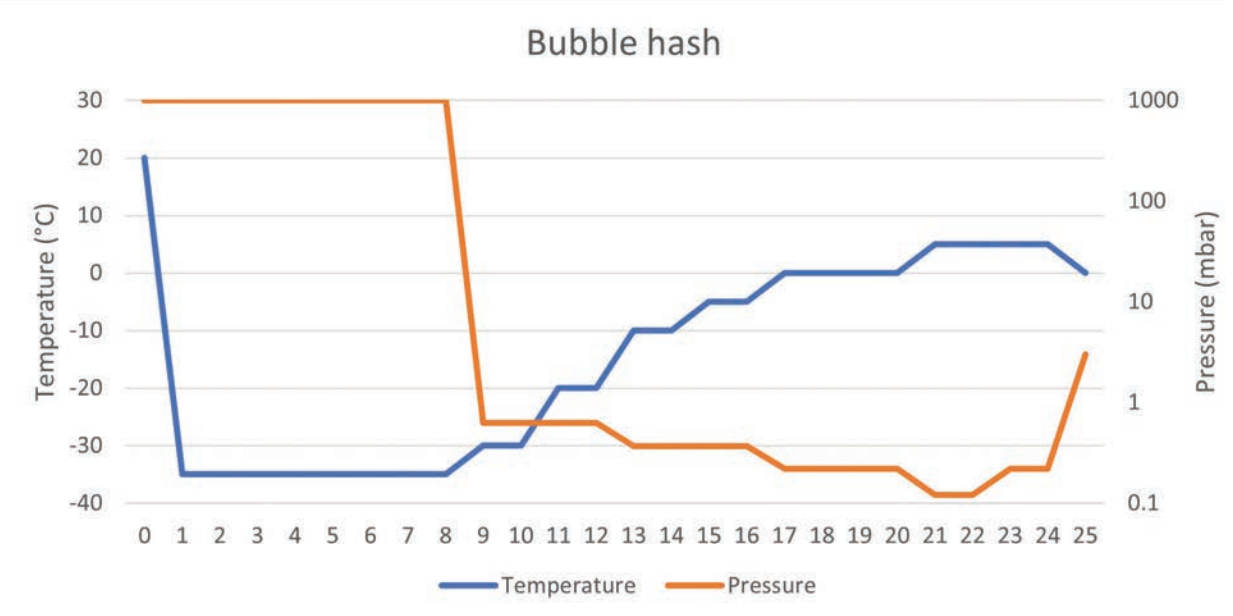
Example Parameter Set:

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-35
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	3
Maximum Shelf Temperature	10

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-30	120	0.63
2	-20	120	0.63
3	-10	120	0.37
4	-5	120	0.37
5	0	240	0.22
6	5	240	0.22



Meat (1-2 cm thickness)

Freezing Temperature <-35°C, 8h.Main Drying Pressure 1.65 to 0.37 mbar.
Final Drying Pressure 0.22 to 0.12 mbar.
Main Shelf Temperatures -20 to 10°C in 10°C steps.
Final Drying Shelf Temperature 20°C.
Drying Section Times >4h.
Total Process Time 24 to 48h.

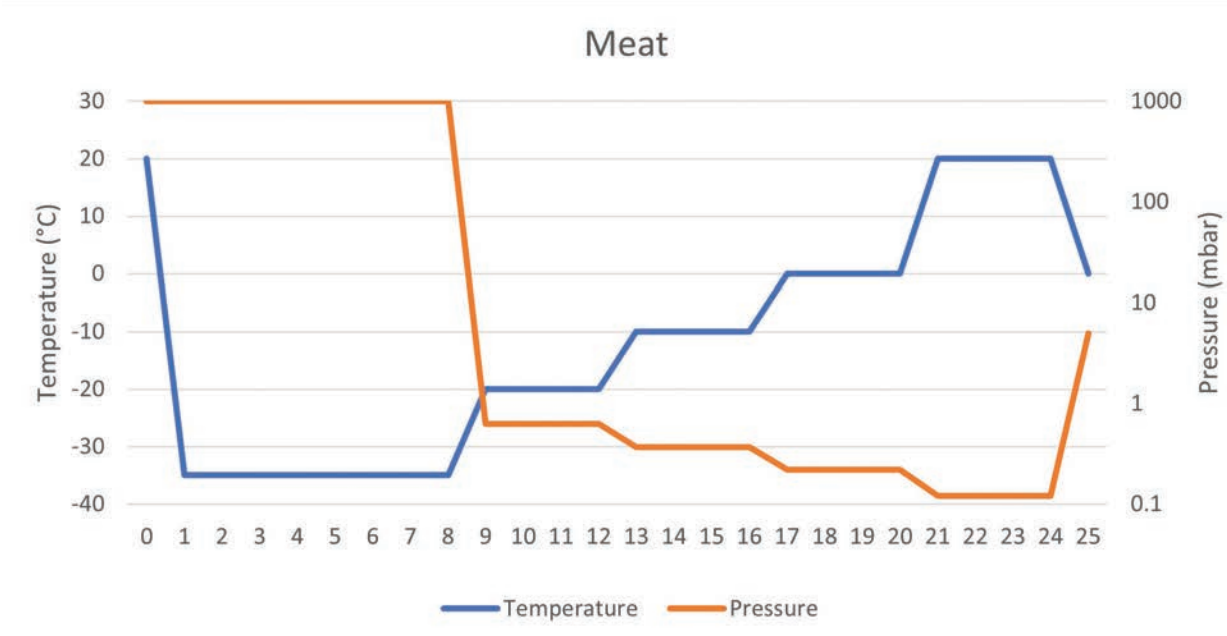
Example Parameter Set:

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-40
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	30

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	240	0.63
2	-10	240	0.37
3	0	240	0.22
4	20	240	0.12



General Botanicals & Plant Samples

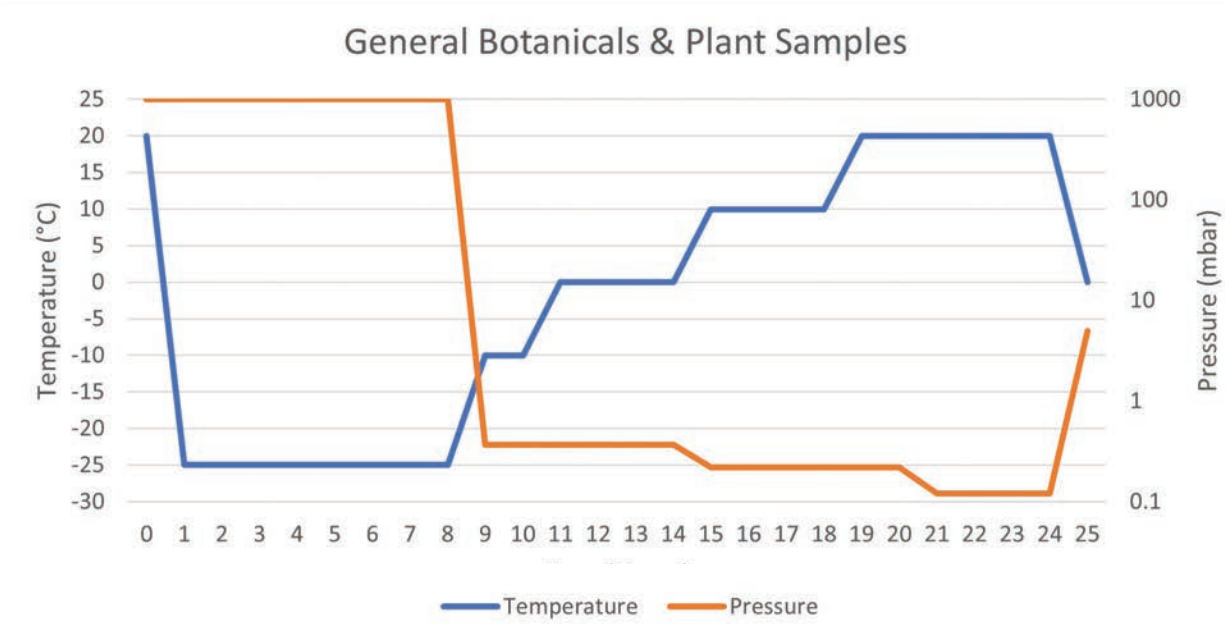
Freezing Temperature <-30°C or max cold, 12h+, generally done in external deep freezer. Time depends on size.
Main Drying Pressure 1.03 to 0.63 mbar.
Final Drying Pressure 0.37 to 0.12 mbar.
Main Shelf Temperatures -30 to 10°C in 5-10°C steps.
Final Drying Shelf Temperature 10-30°C depending on temperature tolerability.
Drying Section Times 4-8h.
Total Process Time 1 day to 1 week depending on size.

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-25
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	30

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-10	120	0.37
2	0	240	0.37
3	10	240	0.22
4	20	360	0.12



Soil / Sediment Samples

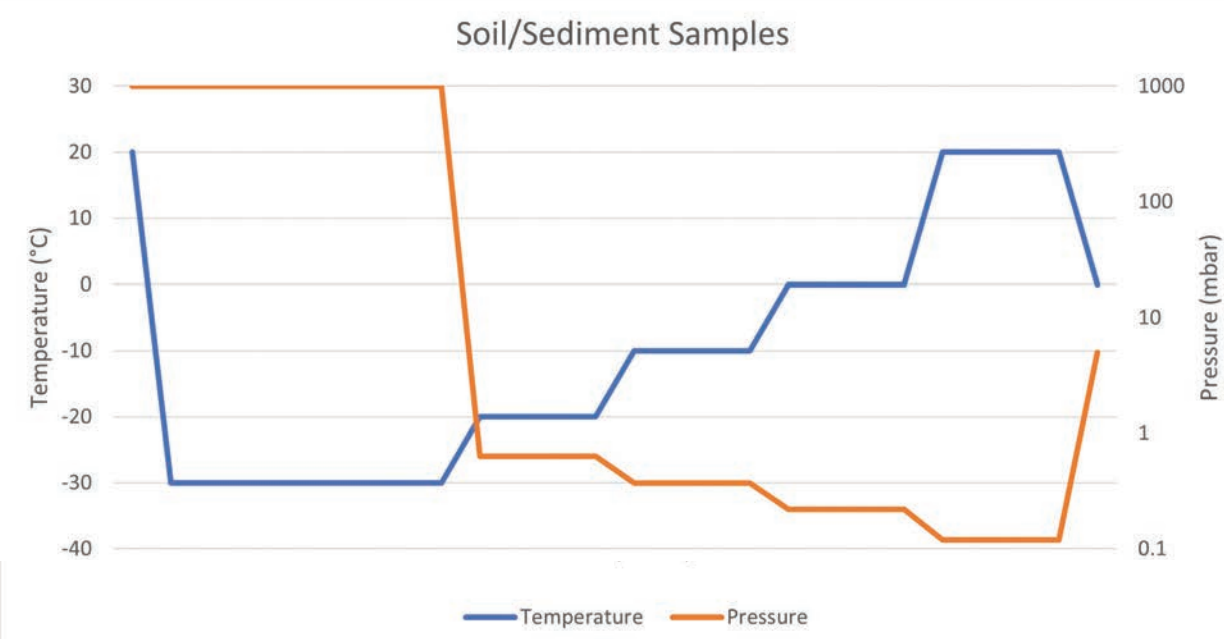
Freezing Temperature <-30°C, 8h.
Main Drying Pressure 0.65 to 0.22 mbar.
Final Drying Pressure 0.22 to 0.12 mbar.
Main Shelf Temperatures -20 to 20°C in 10°C steps.
Final Drying Shelf Temperature 30°C.
Drying Section Times >4h.
Total Process Time 24 to 48h.

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-30
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	30

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	240	0.63
2	-10	240	0.37
3	0	240	0.22
4	20	240	0.12



Bacteria

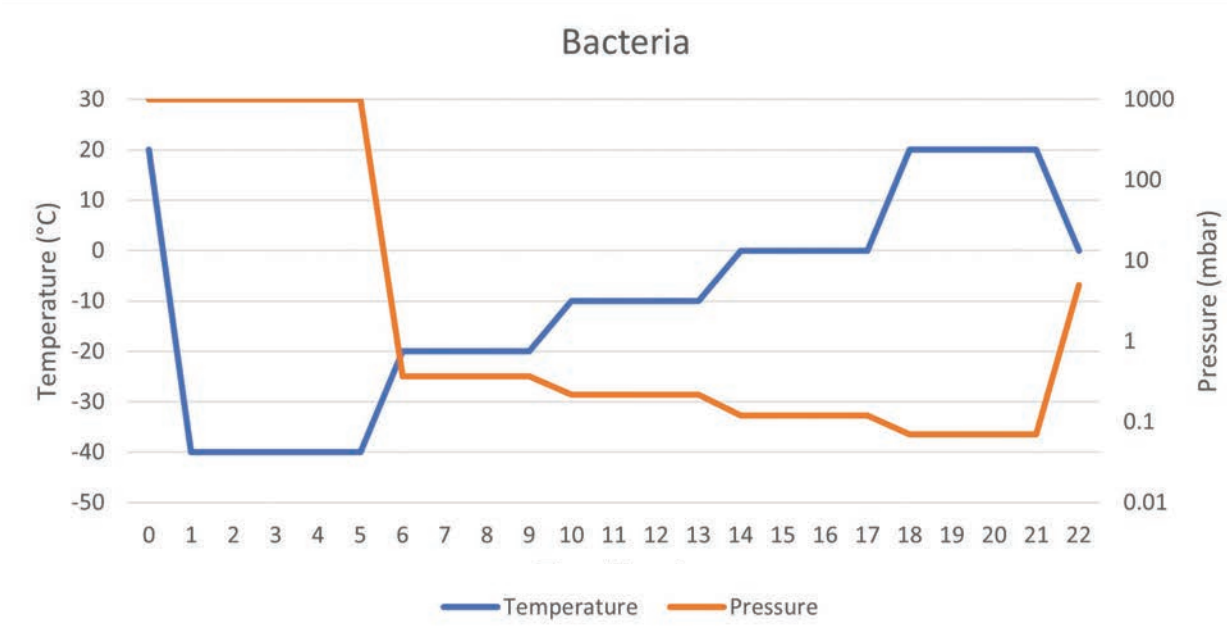
Freezing Temperature <-35°C or max cold, 4-8h.
Main Drying Pressure 0.63 to 0.22 mbar.
Final Drying Pressure 0.12 to max vacuum.
Shelf Temperatures -30 to 0°C in 5°C steps.
Drying Section Times 4h.
Total Process Time 24-48h.

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-40
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	30

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	240	0.37
2	-10	240	0.22
3	0	240	0.12
4	20	240	0.07



HPLC Samples

Freezing Temperature <-40°C, 8h.
Main Drying Pressure 1.03 to 0.22 mbar.
Final Drying Pressure 0.12 to max vacuum mbar.
Main Shelf Temperatures -20 to 20°C in 10°C steps.
Final Drying Shelf Temperature 20°C.
Drying Section Times >2-4h.
Total Process Time 24.

Process Parameters:

Parameter	Value
Freeze Time	480
Freeze Temperature	-40
Evacuation Time	5
Vacuum	1
Vacuum High Limit	3
Vacuum Low Limit	0.01
Vacuum Hysteresis	1
Process End Temperature	0
Process End Pressure	5
Maximum Shelf Temperature	30

Drying Ramp:

Step No 1	Shelf Temperature	Time	Vacuum
1	-20	240	1.03
2	-10	240	0.63
3	0	240	0.22
4	20	240	0.12