Analysis of Seed Sanitization, Rapid Freezer Proficiency, and Veg-03 Water Verification

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Nomenclature

cm	=	Centimeter
HCL	=	Hydrochloric acid
IMA	=	Inhibitory mould agar
mL	=	Milliliter
m^2	=	Meters squared
TSA	=	Trypticase soy agar
Veggie	=	Vegetable Production System

I. Seed Sanitization

Seed sanitation is an important step when sending plants to the International Space Station. The sanitation needs to ensure that the flight seeds are sanitized enough to be free of microbial contaminants yet not over-sanitized to the point that the seed is damaged and germination is hindered. The levels of microbial contaminants and germination rates once sanitized differs between all plants. In order to determine the optimal level of sanitization, a sanitization test was performed with potential flight seeds of both tomato and pepper plants. Hatch tomatoes, NuMex tomatoes, Bulgarian Carrot peppers and Pompeii peppers were chosen as the flight seed candidates for due to their size and levels of fruit production. Space for plant growth on the ISS is limited. When deployed the Vegetable Production System (Veggie) has a growing area of 0.16 m^2 and a height of 45 cm.[3] Due to the limited space on the ISS, the plants need to be small enough to not outgrow Veggie. In addition, the plants should have a high fruit content as a limited amount of plants can be grown on the ISS at the same time and so the more fruits the plants produce, the more vitamins and fresh food the astronauts can consume. Tomato and pepper plants were chosen for this experiment as potential flight seeds because both plants harbor key vitamins needed for long term space travel.[1] Studies have shown that the vitamin B and C levels in pre-packaged food depletes over time and so plants that are high in these vitamins will be crucial for long term space exploration.[1] In addition to vitamin content, taste was also considered. While in microgravity, some astronauts experience a phenomenon in which their food, even the spicy food, tastes bland. This phenomenon is often attributed to the astronauts' "stuffy head."[2] When in microgravity, blood is centered most in the upper part of the body and so the tongue is bloated hindering the taste buds. Because of this, food that would be considered too spicy on Earth is often favored by astronauts when in microgravity. This is why the Bulgarian Carrot and Pompeii peppers were chosen, they are considered hot and a great counter to the often bland prepackaged food.

The procedures for sanitization were derived from the Veg-03 sanitization method, with minor adjustments. Seeds sanitized in Veg-03 were sanitized with .5 mL HCL. For this study, the seeds were split up into two trials for each seed type. One trial was sanitized with .5 mL HCL and the other with .75 mL HCL and microbial analysis and germination rates were compared. The higher levels of HCL added to the ball jars results in a higher amount of chlorine gas produced. The chlorine gas is the active chemical that surface sanitizes the seeds.

Image 1. *Picture of 50mm petri dish containing 100 seeds picked up by tweezers to be placed on top of 50mL inverted flask inside of the ball jar.*

Hatch and NuMex sanitization procedure

Hatch and NuMex tomato seeds were obtained and 200 seeds of each tomato species were counted. The seeds were then placed in four plastic 50 mm petri dishes, resulting in two petri dishes with 100 seeds of Hatch peppers in each dish and two petri dishes with 100 seeds of NuMex peppers. The petri dishes were then set aside. Next, four glass ball jars were placed under a fume hood and a 50mL inverted flask was placed inside each one. Using a manual pipette, 30 mL of bleach was added to each ball jar and the lid was secured. The next portion of the procedures occurred quickly in order to prevent a great amount of chlorine gas from escaping the ball jar. .5 mL of concentrated HCL was carefully added with an automatic pipette, immediately after, the petri dish containing 100 NuMex seeds was placed on top of the inverted beaker with tweezers and the ball jar was shut. This step was repeated once more, this time with a petri dish containing Hatch seeds. Next the pipette used for HCL was set to .75 mL and the above steps were repeated, this time.75 mL HCL was added rather than .5 mL to the two remaining ball jars. The treatments that used .75 microliters were referred to as "750" therefore Hatch tomato seeds that were treated with .75 mL HCL were called "Hatch 750," and NuMex seeds were referred to as "NuMex 750." The jars were left for one hour to be sanitized by the chlorine gas. After the elapsed time the jars were opened in the order that they were shut. The petri dishes were removed with autoclaved tweezers and new petri dish lids were placed on top. The petri dishes were then labeled and left to off-gas overnight. In the morning the petri dishes were parafilmed and placed in a refrigerator set to 4° C.

Twenty seeds of each treatment were used for microbial analysis. The treatments were Hatch 500, Hatch 750, Hatch control, NuMex 500, NuMex 750 and NuMex control. The control groups were taken directly from the vendor bag that the peppers came in and placed in a 50 mm plastic petri dish. Microbial analysis tested for any bacterial or fungal growth on the surface of the seeds. TSA and IMA plates were used to achieve this. Trypticase soy agar or "TSA" is a

universal, non-selective medium that provides the nutrients for both gram positive and gram negative bacteria to thrive. Inhibitory mould agar or "IMA" provides an optimal growth environment for fungi and contains chloramphenicol, a selective agent that prevents most bacterial growth. Seeds were plated directly on the agar.

TSA and IMA seed plating procedure

Five seeds of each treatment were plated on each agar type with two replicates of each. When plating the seeds, the autoclaved tweezers were sanitized with 70% ethanol and heated with an incinerator before coming in contact with a new seed. The plates were then placed in a 35° C incubator and monitored each day. Final results for IMA were recorded on day five and final results for TSA were recorded on day three.

Germination test procedure

A germination test was performed for each treatment to ensure that the sanitization did not impede the germination rates. Three square petri dishes were obtained and double-lined with filter paper. Nanopure water was then poured over the filter paper, enough to where the paper was saturated and there was some (approximately 2 mL) free water remaining. Seeds were then placed on the petri dishes which were then sealed with parafilm and placed in an environmental chamber. Each petri dish had two different trials which were separated by the middle of the petri dish. After 5 days the results of the germination tests were recorded.

Image 2. *Picture of the end stage of Hatch tomato seed germination test.*

Hatch Control	Hatch 500	Hatch 750	NuMex Control	NuMex 500	NuMex 750
8/10 seeds	6/10 seeds	7/10 seeds	10/10 seeds	10/10 seeds	7/10 seeds

Chart 1. *Chart of germination rates. 10/10 seeds means all seeds germinated, 0/10 seeds would mean that there was no germination.*

Sanitized Hatch seeds were very similar in growth, with the .75 mL treatment doing slightly better. NuMex seeds sanitized with .75 mL HCL had a noticeably lower germination rate than the other NuMex treatments, yet the germination rate was not inferior to the Hatch seed germination rate with the same treatment.

IMA RESULTS

Hatch Control	Hatch 50	Hatch 750	NuMex Control	NuMex 500	NuMex 750
6/10 seeds	2/10 seeds	0/10 seeds	10/10 seeds	1/10 seeds	0/10 seeds

Chart 2. *Chart of IMA growth rates. 0/10 seeds means there was no fungal growth on any of the seeds, 10/10 means that all seeds had fungal growth.*

TSA RESULTS

Hatch Control	Hatch 50	Hatch 750	NuMex Control	NuMex 500	NuMex 750
10/10 seeds	10/10 seeds	9/10 seeds	10/10 seeds	3/10 seeds	0/10 seeds

Chart 3. *Chart of TSA growth rates. 0/10 seeds means there was no bacterial growth on any of the seeds, 10/10 means that all seeds had bacterial growth.*

All treatments with .75 mL HCL resulted in no fungal growth while the treatments with .5 mL resulted in slight fungal growth and the controls of both species had the most growth of the three treatments. Bacterial growth was also less with the .75 mL HCL yet the Hatch seeds still had a large amount (90%) of bacterial growth.

The sanitization method with .75 HCL was determined to be the preferred method for the Hatch and NuMex seeds as it resulted in the lowest amount of fungal and bacterial growth with acceptable germination rates. NuMex seeds sanitized with .75 mL of HCL did favorable to the Hatch seeds as there was no bacterial or fungal contamination and the germination rates were the same. Because of this, NuMex seeds have been chosen as the flight seed candidates.

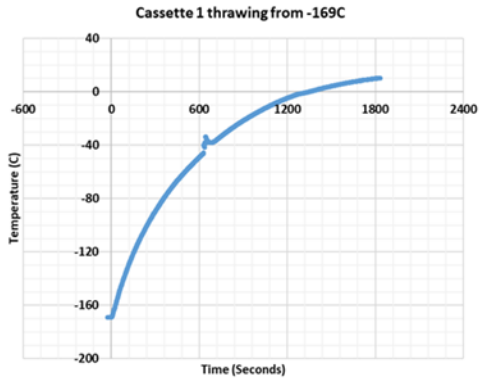
Pepper seed sanitization

Given the success of the .75 mL HCL sanitization method, two pepper seed flight candidates were tested with the .75 sanitization method, the .5 mL HCL sanitization method was not used. 30 seeds of each type, Bulgarian Carott and Pompeii, were sanitized with .75 HCL using the same methods as the tomato seeds. Microbial analysis was preformed using the same procedures and no bacterial or fungal growth was found. A germination test is in progress, results and potential flight test candidates are to be determined.

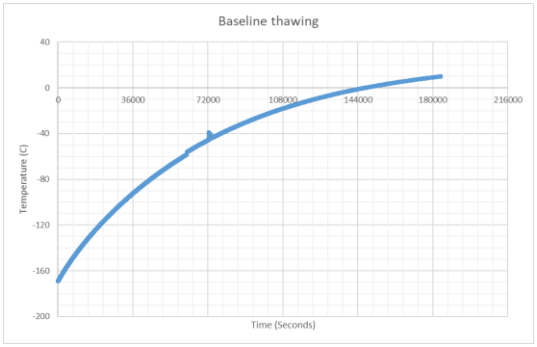
II. Rapid Freezer

The rapid freezer is a valuable tool that allows astronauts to freeze biological samples to temperatures as low as -180° C. The technique of fast freezing by use of a rapid freezer was created as a much safer alternative to flash freezing samples in liquid nitrogen, yet it maintains the integrity of the sample in the same way. A cartridge fits easily into the flash freezer and is able to hold as many as four cassettes, each of which are made to hold biological samples.

In order to fully understand the change in temperature per change in time, the temperature changes of the cartridge and cassettes were recorded by temperature sensors. The cartridge and cassettes were recorded thawing from their lowest temperatures. This is valuable as it is important to note if one component of the rapid freezer takes longer to thaw than other components. Probes were placed in a cartridge and cassettes in the rapid freezer and then frozen. The data assessed how long it took each component to reach goal temperatures of -80° C, -40° C and -20° C. Key temperatures along with their corresponding times were recorded. Temperature change of a single cassette, with the temperature sensor pressed against the inside wall, was assessed from its lowest recorded temperature of -162.9° C. It reached -79.6° C after 357 seconds, -38.3 C after 637 seconds, -20° C after 923 seconds and took a total of 4730 seconds to reach its highest recorded temperature of 20.1° C. The cartridge (or baseline) began at its lowest temperature of -169° C at time 0 and reached its highest temperature of 10° C at 183926 seconds after oscillating between 10° C and 9.9° C for 259 seconds within the 183669 and 183928 second marks. It reached -80° C after 44344 seconds, -40° C after 78061 seconds, and -20° C after 104772 seconds. The large variation in time suggests that the cassettes raise their temperature faster than the carrier.



Graph 1. *Graph of changes of temperature over time with the probe placed in a cassette.*



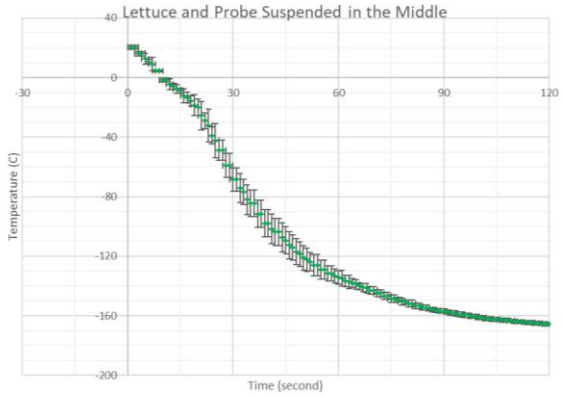
Graph 2. *Graph of changes of temperature over time with the probe placed in a carrier.*

Image 3. *Picture of trial 1 probe and leaf that was suspended in cassette.*

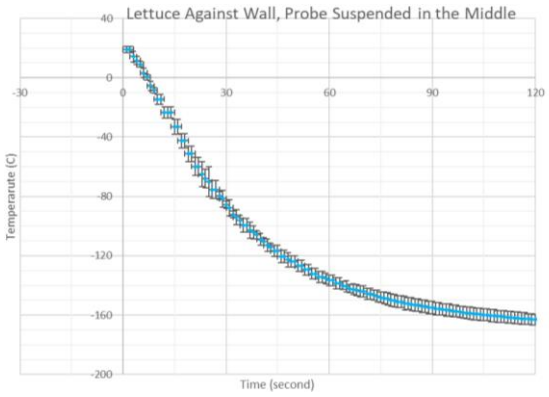
Given that there is a large temperature variation between the carrier and the cassettes, the next step was to determine if there was a variation of temperature in the cassettes themselves. When the astronauts place their biological samples in the rapid freezer it is important to know exactly what temperature the samples are. If there is a temperature displacement in the cassettes and a part of the cassette stays warmer than the rest then the biological sample that may be subjected to the warmer temperatures may be subject to contaminants or insufficient preservation. Small circles 1/4'' in diameter (as seen in Image 3.) were made by use of a handheld single hole punch. Trial 1 assessed the probe temperature with the probe and leaf attached and suspended in the middle of the cuvette. This was achieved by using scotch tape to attach the probe to the leaf and then lab tape to attach the leaf and probe to the tape "bridge" which held the probe in the middle of the cuvette. Trial 2 assessed the temperature of the probe when it was suspended in the middle of the cuvette by a tape "bridge" across the inside of the cuvette. The leaf was then taped against the inside wall of the cuvette, not touching the probe

The data set for each trial began when the first cuvette reached a temperature below 22° C and the cuvettes were then frozen to their lowest temperatures. The average time it took for the cuvettes of each trial to reach goal

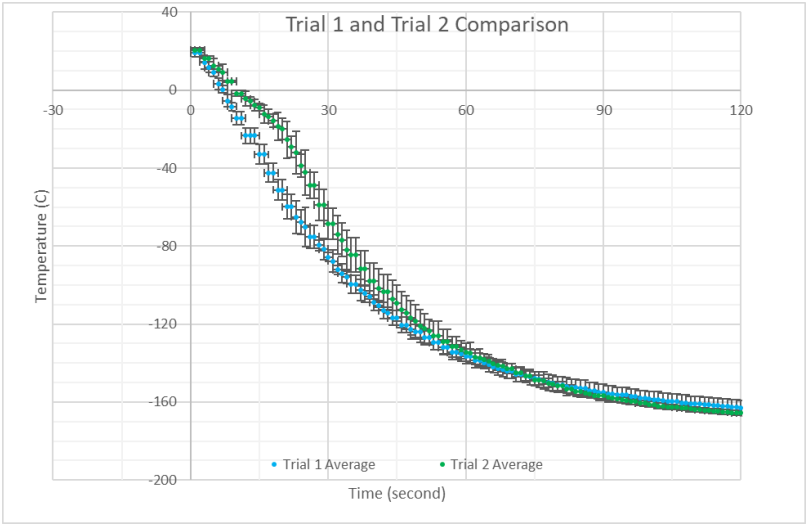
temperatures was recorded. Trial one took 11 seconds to reach -20° C, 16 seconds to reach -40° C and 28 seconds to reach -80° C. Trial two took 20 seconds to reach -20° C, 24 seconds to reach -40° C and 33 seconds to reach -80° C. Graph 5 is a graph comparing the two averages. As seen on the graph, the averages of each trial are close in temperature per unit of time and the error bars of each trial are overlapping. The results of this experiment show no statistically significant difference between the average temperatures of each trial. This means that the temperature inside the cuvettes are similar and there is no statistically significant change in temperature if the biological sample is on the wall or suspended in the middle of the cuvettes.



Graph 3. *Trial 1. Graph of changes of temperature over time with temperature sensing probe and biological sample suspended in the middle of the cassette.*



Graph 4. *Trial 2. Graph of changes of temperature over time with the biological sample pressed against the wall and temperature sensing probe suspended in the middle.*



Graph 5. *Comparison graph of trial 1 and trial 2.*

III. Water Evaporation Test

When plants are grown on the ISS, pictures of the plants are often received three days after they are taken on the Space Station. Because of this, it is important to ensure that the amount of water the plants should receive every day is the correct amount for the plant to thrive. If the astronauts are instructed to give the plants too little water, the three days it takes for the pictures of the dehydrated plants to reach the project scientists at NASA may cause irreversible damage o the plants. To try to mitigate this, a water evaporation test was set up to determine if the amount of water that the astronauts have been instructed to give the Veg-03 seedlings was enough. This would be determined by a simple qualitative and quantitative analysis. The beakers would be qualitatively assessed by determining if there is any "free water" in the jar and quantitatively assessed by determining the mass of water left in the beaker after three days.

30 mL of water was placed in a 200 mL beaker containing arcelite filled up to the 75 mL mark. The arcelite was then left for three days to evaporate and the changes in weight of each beaker was recorded.

Figure 5. *Comparison of dry arcelite (right) to the test arcelite (left) after three days.*

Figure 6. *Image of "free water" in the beaker. This water has not yet been absorbed by the arcelite, which indicated that the arcelite is saturated with water.*

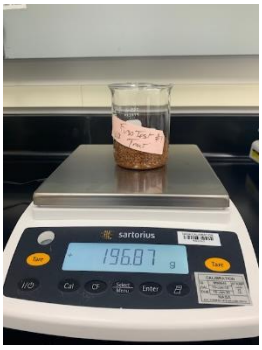


Figure 7. *Progress image of "Test 3" after three days.*

	Initial Weight (grams)	Weight after 3 days (grams)	Difference (mL)
Test 1	204.72	196.87	7.85
Test 2	213.17	204.44	8.73
Test 3	207.21	198.87	8.34
Test 4	210.27	201.02	9.25

Graph 4. *Initial weight, weight after three days and the difference in weight.*

The qualitative data after three days showed that the arcelite had plenty of water and was in no risk of drought. The quantitative data, as seen in graph 4, shows that not all of the water was evaporated. 30 mL of water was added and approximately only one third of that was evaporated after day three. The mL lost was determined as one mL of water is equal to one gram. Given these results the astronauts were not instructed to water the plants more.

References

[1]Cooper, M., Perchonok, M., & Douglas, G. L. (2017, June 09). Initial assessment of the nutritional quality of the space food system over three years of ambient storage.

[2]Dunbar, B. (2018, March 07). A Matter of Taste

[3]Massa, G. D., & Levine, H. G., "Veg-03," ISS Program Science Office