Detecting Fungal Infections in Almonds

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Abstract

This protocol is based on the study done by two members of the Biological and Agricultural Engineering department at UC Davis which analyzes the ability of infrared spectroscopy to detect fungal infections in almonds. The methods section of this article was adapted to fit the protocols.io format.

Source:

“Detection of fungal infection in almond kernels using near-infrared reflectance spectroscopy”. Pei-Shih Liang, David C. Slaughter, Alejandro Ortega-Beltran, Themis J. Michailides. Biosystems Engineering, Volume 137, September 2015, Pages 64–72

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Before start

Before starting the protocol it is important to have gathered almond kernel samples from different harvest seasons to better understand the pattern of infection for this specific crop.

Protocol

Step 1.
Manually select almond kernel samples that are pasteurized and shelled.
As mentioned in the 'Before Start Instructions', the almond kernels should be collected from multiple harvest cycles. These particular samples were obtained from the 2012 and 2013 almond seasons. The samples should be shelled and should not have any type of physical damage or any marks.

Step 2.
Record weight and size of almond kernels

Step 3.
Separate the samples into two groups.

Out of the 550 almond kernels collected 460 of those will be infected with two types of fungi that commonly affect almond crops. 230 will be infected with *A. flavus* and another 230 will be infected with *A. parasiticus.* The remaining 90 kernels will remain uninfected and will serve as a control group.

Step 4.
Obtain A. flavus and A. parasiticus isolates to infect almonds.

These two types of fungi were chosen due to their well known colonization on the surface of almond kernels.

Step 5.
Prior to inoculation, take the fungi isolates and grow them in culture.
Use 5% reconstituted tomato juice agar, in this case it will be V8 juice agar, and grow each isolate for 6 days at a temperature of 31 °C in a dark environment.

Step 6.
Once fungal isolates have been grown, conidia is collected using a sterilized cotton swab and is deposited in sterile deionized water. A haemocytometer will be used to quantify the water solution with conidia and it will then be diluted to a concentration of 10^5 spores per ml^{-1}.

Step 7.
Create incision on the bottom right of almond kernels using a B-D® 16G1 PrecisionGlide®Needle. This incision serves as the point of infection where the conidia in water would be placed.

Step 8.
After making the incision on the surface of the almond, the kernels will be sterilized by submerging them in 10% bleach solution for a period of 7 minutes.

Step 9.
The kernels will then be rinsed three times with sterile deionized water and will be submerged a second time into a Dichloran solution for 2 seconds.

NOTES

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The Dichloran solution will be 0.2% in 95% ethanol.

Step 10.
Remove moisture from surface of almonds by blotting them and move them to a biological safety cabinet for a period of 10 to 15 minutes.

Step 11.
Inoculate each almond with 5 µl of the fungi solution.
As mentioned earlier, almonds will be in two separate groups: infected and control. The infected almonds will be inoculated with either A. flavus or A. parasiticus.

Step 12.
Once the almonds have been inoculated, add deionized water to the container where they are located and incubate them for a period of 7 days at a temperature of 31° in a dark place.

NOTES

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The water that is added to the bottom of the container containing the almonds helps promote an environment where the fungi can grow.

Step 13.
Once the incubation period is over, wash almonds with deionized water to remove any traces of fungi spores from the surface.

NOTES

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The deionized water used in this step contains 0.05% Tween-20 which is a solution that works as a type of cleaner for the almond surfaces.

Step 14.
Place almond kernels in a fume hood for a period of 12 hours. Once the 12 hours are up keep kernels

at a constant temperature of 2.6°C.

Step 15.
Take almond and put it under the spectrophotometer, then place black optic mask in the space between these two.

NOTES
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The black optic mask allows us to control the size of the area being scanned by the spectrophotometer.

Step 16.
Scan the surface of the almond kernel using the spectrophotometer, for each individual almond kernel take 32 scans of the face across a wavelength range of 400 nm to 2500 nm, in 2-nm increments.

Step 17.
Use statistical analysis software to analyze the data gathered from all three groupings of almonds.