FSHN0407

UF UNIVERSITY of FLORIDA
IFAS Extension

A Method for Detecting Fungal Contamination in Paperboard Cartons[1]

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Contamination of refrigerated juice products in gable-top cartons may occur by filamentous fungi that are present in the paperboard. The method presently used by the food industry to assess the amount of fungal contamination in the paperboard carton material is cumbersome and inefficient (1). To ascertain what types of fungal organisms are present in paperboard containers used to hold foods and beverages with an extended shelf-life, a direct-plating method was developed which is efficient, accurate and utilizes little in the way of time and resources (2). This method incorporates routinely used mycological techniques, including medium and incubation procedures which insure that conditions for fungal growth are optimal and results will be reliable.

Method of Analysis

1. Randomly select 2 or 3 cartons from each lot of unformed paperboard cartons to be assayed. Samples used for analysis are from the lots to be used for beverage/food fills. Sample number can vary: a larger number of sample pieces yields a greater diversity and number of organisms and better reproducibility.

2. Prepare agar plates (PDAN agar: Commercial potato-dextrose agar [non-acidified] + novobiocin [0.04g/L] added before autoclaving to prevent competition by bacterial contaminants). After tempering to 50°C, pour medium into sterile Petri plates and allow it to solidify.

3. Using clean, disposable gloves, cut narrow strips (approximately 1 cm wide) from all sides of the carton including the fifth panel (folded side-seam), gable area, bottom fold area and mid-carton sections (Fig. 1). Use scissors with blades that were washed with hot soap and water, rinsed several times and dipped in lab grade isopropanol.

4. Cut strips into small (about 1 cm^2) pieces.

5. Place cut pieces into a sterile glass Petri dish until all are collected.

6. Transfer cut pieces from the Petri dish to a sterile glass jar (a large-mouth milk dilution bottle works well).

7. Add a 5% solution of household bleach (5 mL bleach plus 95 mL sterile water) to cover all the

1. This document is FSHN04-07, one of a series of the Food Science and Human Nutrition Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Publication date: August 2004. Please visit the EDIS Web site at **http://edis.ifas.ufl.edu**.
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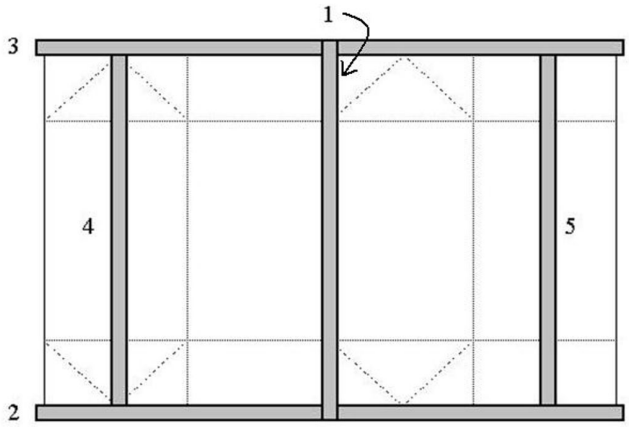

Figure 1. An unformed, flat gable-top carton is depicted. Dark gray strips are approximately 1 cm wide and are cut from the carton with sterile scissors. Strips are then cut into pieces approximately 1 cm^2. Strip 1 is the side-seam known as the "fifth panel." Strips 2 and 3 are the package top and bottom. Strips 4 and 5 are in the middle of two side panels.

sample pieces and allow all surfaces to be exposed to the cleaning solution.

8. Agitate pieces in the solution for 3 minutes.

9. Decant the bleach solution leaving the pieces in the container. Aseptically add enough sterile, distilled water to the bottle to cover all pieces and agitate the pieces for one minute. Decant rinse water, add more. Repeat rinse for a total of 5 rinses.

10. After the last rinse, aseptically transfer the pieces to another sterile Petri dish for ease in handling the small samples.

11. With sterile gloves and sterile forceps, take the pieces from the dish and push into solidified PDAN (PDA + novobiocin) plates so that the sample pieces stand perpendicular to the agar surface (Fig. 2).

12. Number of pieces per plate will vary with sample size. Place up to 15 pieces in a 100 x 15 mm plate. Use as many plates as needed to accommodate all carton pieces.

13. Label plates and incubate 7-10 days at 23-25°C.

14. At the end of incubation time, count fungal colonies growing from sample pieces to evaluate contamination (Fig. 2). Report the number of fungal

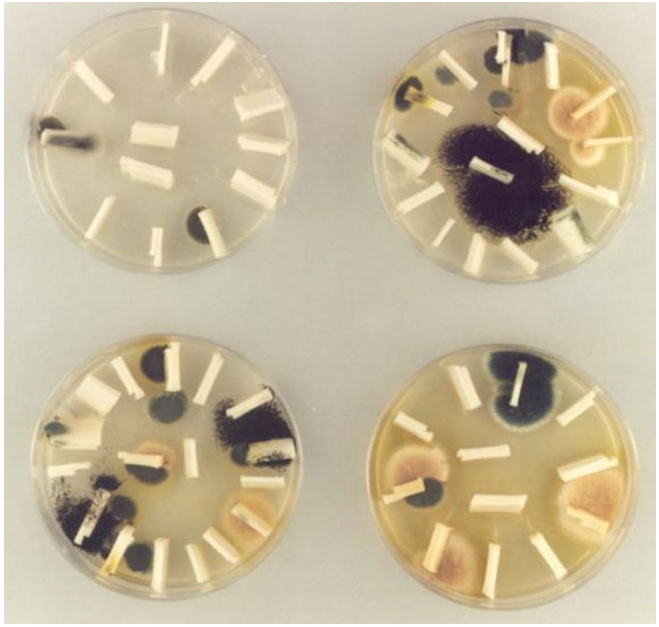

Figure 2. Petri plates of PDAN are shown with 1 cm^2 pieces of carton inserted into the agar perpendicular to the surface. Plates were incubated 7 days at 25°C. Note the number of fungal colonies growing from the carton pieces. Each colony is assumed to represent a single fungal propagule.

propagules per number of grams of carton pieces assayed, or per the total surface area assayed.

Discussion

The method described above is less cumbersome, more sensitive, uses fewer resources and allows fewer opportunities for contamination (by organisms other than those in the paperboard sample) to occur, than the method presently used by the food industry for isolating fungi from paperboard. Following the outlined procedures, personnel associated with quality assurance will have the capability to evaluate complaints of contamination in the product as well as to periodically assess the cleanliness of the containers used for packaging. Sample incubation times may be shorter for more rapidly growing organisms and/or if contamination is extreme. Since fungal propagules are not evenly distributed within the fibers of the paperboard container, some samples may not contain fungi. Therefore, a relatively large sample size (10+ g) will most accurately determine the amount of contamination in the packaging. Data generated over time can be used to determine acceptable results for individual package applications.

References

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