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Comparison of New and Traditional Culture-Dependent Media for Enumerating Foodborne Yeasts and Molds

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ABSTRACT

Fifty-six foods and food ingredients were analyzed for populations of naturally occurring yeasts and molds using Petrifilm rapid yeast and mold (RYM) count plates, Petrifilm yeast and mold (YM) count plates, dichloran rose bengal chloramphenicol (DRBC) agar plates, acidified potato dextrose agar (APDA) plates, and dichloran 18% glycerol (DG18) agar plates. Colonies were counted after incubating plates for 48, 72, and 120 h at 25°C. Of 56 foods in which either yeasts or molds were detected on at least one medium incubated for 120 h, neither yeasts nor molds were detected in 55.4, 73.2, 21.4, 19.6, and 71.4% of foods plated on the five respective media and incubated for 48 h; 10.7, 14.3, 3.6, 1.8, and 19.6% of foods were negative after 72 h, and 3.6, 1.8, 0, 0, and 0% of foods were negative after 120 h. Considering all enumeration media, correlation coefficients were 0.03 to 0.97 at 48 h of incubation; these values increased to 0.75 to 0.99 at 120 h. Coefficients of variation for total yeasts and molds were as high as 30.0, 30.8, and 27.2% at 48, 72, and 120 h, respectively. The general order of performance was DRBC = APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 when plates were incubated for 48 h, DRBC > APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 when plates were incubated for 72 h, and DRBC > APDA > RYM Petrifilm = YM Petrifilm > DG18 when plates were incubated for 120 h. Differences in performance among media are attributed to the diversity of yeasts and molds likely to be present in test foods and differences in nutrient, pH, and water activity requirements for resuscitation of stressed cells and colony development.

Knowledge of the number of yeasts and molds in foods is useful when assessing the quality and extent of spoilage as well as predicting shelf life. In addition, the presence of potentially mycotoxigenic molds may indicate a public health hazard. Mycological evaluation of foods and food ingredients has become routine in quality assurance programs, and several methods have been recently developed to determine yeast and mold counts within shorter times compared with those required for traditional culture methods.

Among the first nontraditional culture methods demonstrated to perform well for enumerating yeasts and molds in a wide range of foods is the Petrifilm yeast and mold (YM) count plate (3M Food Safety, St. Paul, MN) *(10, 11, 22, 31)*. Studies have shown that YM Petrifilm plates are equivalent to traditional agar media for enumerating yeasts and molds in dairy products such as cheeses *(29, 34)* and yogurt with or without fruit *(34)*. A collaborative study was done to compare YM Petrifilm to traditional mycological media for enumerating yeasts and molds in six foods artificially inoculated with one or two yeasts or molds representing four fungal genera *(22)*. Based on that study, the YM Petrifilm method was adopted as a first action method by AOAC International *(22)*.

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A rehydratable dry medium sheet system (Compact Dry) was developed by Nissui Pharmaceutical Co. Ltd. (Yuki, Ibaraki, Japan). Although dichloran 18% glycerol (DG18) agar, YM Petrifilm plates, and Compact Dry plates appeared to be acceptable for enumerating yeasts and molds in 97 test foods, overall dichloran rose bengal chloramphenicol (DRBC) agar recovered higher numbers of fungi *(8)*.

The Simplate method has been described by the manufacturer (BioControl Systems, Inc., Bellevue, WA) as having the major advantages of a relatively short incubation time (48 h) and the ability to detect a wide range of cell numbers using a single test. Spangenberg and Ingham *(29)* found that the results of the Simplate method are less highly correlated than are those of potato dextrose agar (PDA) supplemented with chlortetracycline, DRBC, and YM Petrifilm plates for enumerating yeasts and molds in low-moisture, part-skim mozzarella cheese. In another study, there was no equivalence in counts between Simplate and DRBC, DG18, PDA plus chlortetracycline and chloramphenicol, and YM Petrifilm plates for enumerating yeasts and molds in a composite of 11 foods, with significant differences in counts for most foods analyzed *(31)*.

NeoFilm for yeast and mold enumeration (Neogen Corp., Lansing, MI) was recently developed, and its performance was compared with that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual*

(FDA-BAM) reference method *(33)* for enumerating yeasts and molds in five types of foods *(1)*. A high level of agreement was observed when plates were incubated for 48 or 72 h at 25°C. Performance varied, depending on the type of food and composition of the diluent.

Another recent advancement in food mycology methodology is the development of the Petrifilm rapid yeast and mold (RYM) count plate (3M Food Safety). RYM Petrifilm plates are described as being easier to use than traditional methods, having proven reliability versus DRBC, DG18, and acidified PDA (APDA), and enabling actionable results within incubation times of 48 to 60 h. Chandrapati and Nordby *(14)* did a comparative yeast and mold enumeration study using RYM Petrifilm plates and reference media recommended by Tournas et al. *(33)* in the FDA-BAM. Results obtained by plating 75 naturally contaminated food matrices on RYM Petrifilm plates and incubating for 48 to 60 h were reported in an abstract of the study to be statistically comparable to results using DRBC and DG18 incubated for 5 days. In contrast, a poster developed from the abstract indicated that 42 foods were tested, and samples with very low or undetectable indigenous fungal populations were artificially inoculated with five yeast species and three mold species before comparative analysis. The number of foods inoculated with yeasts and molds was not stated.

In a subsequent study, RYM Petrifilm was compared with traditional agar media for enumerating yeasts and molds in 10 food matrices as part of an AOAC Research Institute validation process *(16)*. Foods not naturally contaminated were artificially inoculated with a yeast or mold, although the number of foods inoculated was not stated. The authors concluded that RYM Petrifilm demonstrates reliability as a rapid alternative for the enumeration of yeasts and molds in as little as 48 h. In a collaborative study involving 16 laboratories, RYM Petrifilm was compared with the FDA-BAM and International Organization for Standardization (ISO; Geneva, Switzerland) reference methods for enumerating yeasts and molds in eight artificially inoculated frozen ground beef and raw almond samples *(12, 13)*. *Trichosporon mucoides* and *Aspergillus aculeatus* were used as inocula for the ground beef and raw almonds, respectively. No significant differences were observed in the number of yeasts and molds recovered on RYM Petrifilm when counts were made at 48 h versus 60 h or when plates were incubated at 25 or 28°C when compared with reference methods. Counts at incubation times longer than 60 h were not reported. Based on these studies and a precollaborative study *(16)*, the RYM Petrifilm was adopted as a first action method by AOAC International *(13)*. Saengklai et al. *(28)* evaluated the performance of RYM Petrifilm for enumerating yeasts and molds in eight beverage samples; five were naturally contaminated and three were inoculated with heat-treated *Saccharomyces cerevisiae* and *Aspergillus niger* spores. Results compared favorably with counts on chloramphenicol-supplemented plate count agar when plates were incubated at 25 or 28°C for 48 and 72 h. Naturally contaminated foods ($n = 65$) were used by Chandrapati et al. *(15)* to determine the efficacy of RYM Petrifilm for enumerating foodborne yeasts and molds. Recovery was comparable to that on reference media. A validation study showed that recovery of foodborne yeasts and molds on RYM Petrifilm is comparable to recovery on media recommended by the ISO *(2)*.

The use of yeasts and molds grown on mycological media to artificially inoculate foods used in some of the tests to evaluate RYM Petrifilm and traditional media raises concern about the performance of these media in recovering naturally occurring fungal conidia, spores, and hyphae that may be in various states of debilitation caused by low water activity (a_w), extreme acidic or alkaline pH, suboptimal oxygen availability, exposure to temperature extremes, naturally occurring antimycotics, and other stress factors imposed by food and beverage matrices over an extended period of time. Compared with fungal cells freshly harvested from mycological media, stressed cells need to undergo a period of resuscitation under optimum recovery conditions before growth and colony development can occur *(4, 5, 17–19, 24, 25, 30)*.

Comparative evaluation of the performance of RYM Petrifilm plates against other media to accurately enumerate yeasts and molds at incubation times of less than 5 days, the time recommended by Tournas et al. *(33)* in the FDA-BAM, should ideally be done using naturally contaminated foods. If mycological media are evaluated for enumerating yeasts and molds in a food not naturally contaminated, the food should be held for a period of time after inoculation to facilitate exposure of cells to stress conditions before analysis. This was done in studies involving frozen beef patties and raw almonds *(12, 13)*.

The objective of the present study was to compare RYM Petrifilm plates, YM Petrifilm plates, and DRBC, APDA, and DG18 media for their ability to support visible colony formation by naturally occurring yeast and mold contaminants within incubation times of 48, 72, and 120 h at 25°C.

MATERIALS AND METHODS

Foods and food ingredients tested. One hundred sixteen foods and food ingredients were screened for yeast and mold populations. High- and low-a_w raw and processed foods of animal and plant origin were tested. All products were obtained from commercial sources, either directly from manufacturers or at local retail stores. Frozen foods were thawed at 21 to 23°C immediately before analyzing. Hazelnuts, peanuts, walnuts, and sunflower seeds were chopped into coarse pieces in a nut chopper (model HC306, Black and Decker, Towson, MD). Broccoli, lettuce, green onions, and sliced apples were diced or cut into 0.5-cm pieces. Pieces or portions of all other foods and ingredients were analyzed in the forms received from the manufacturers or purchased at retail.

Enumeration methods evaluated. Five methods (media) were evaluated for recovering yeasts and molds: RYM Petrifilm count plates (3M Food Safety), traditional YM Petrifilm count plates (3M Food Safety), DRBC agar (Neogen), APDA (PDA acidified with tartaric acid) (Neogen), and DG18 agar (Neogen). The pH values of RYM Petrifilm and YM Petrifilm plates were 5.3 and 5.4, respectively; the pH of DRBC and DG18 was 5.6 ± 0.1; the pH of APDA was 3.5 ± 0.1.

Plating procedure. Fifty-six of the 116 foods and food ingredients screened for the presence of yeasts and molds

TABLE 1. *Comparison of counts on traditional agar media for recovering yeasts, molds, and yeasts plus molds from 56 foods and food ingredients after incubation for 120 h 25°C without interruption and counts on plates incubated for 120 h but also examined at 48 and 72 h*

Microorganisms	No. of foods or ingredients[a]	No. of samples higher/lower[b]		
		DRBC	APDA	DG18
Yeasts	34	2/6	6/1	2/1
Molds	43	4/3	6/0	3/2
Yeasts + molds	56	6/5	9/1	4/3

[a] Yeasts, molds, and yeasts plus molds were detected in or on 34, 43, and 56 foods and food ingredients, respectively. For foods and food ingredients in which no yeasts or no molds were detected (<10 CFU/g or ml), yeast plus mold populations reflect values for only molds or yeasts.

[b] Values indicate the number of samples out of 34 (yeast), 43 (mold), or 56 (yeast plus mold) foods and food ingredients for which the number of colonies formed on plates incubated for 120 h but also examined for colony formation at 48 and 72 h was significantly higher/lower ($P \leq 0.05$) than the number of colonies formed on plates incubated for 120 h without handling at 48 and 72 h.

contained populations ≥10 CFU/g or ml. All samples were processed as described below and plated on DRBC. Plates incubated for 120 ± 2 h at 25°C were examined for yeast and mold colonies. Triplicate samples of the 56 foods and food ingredients shown to contain yeasts and molds at populations >10 CFU/g or ml were analyzed using RYM and YM Petrifilm plates, DRBC, APDA, and DG18 as recovery media. Samples (25 g) of foods were combined with 225 ml of sterile 0.1% peptone water in a stomacher bag and homogenized in a stomacher (Stomacher 400, Seward Medical Ltd., London, UK) for 2 min. Undiluted homogenates (1.0 ml) and homogenates (1.0 ml) diluted 10-fold in sterile 0.1% peptone were deposited on RYM and YM Petrifilm plates as instructed by the manufacturer. Undiluted homogenates (quadruplicate 0.25-ml samples) and homogenates (0.1 ml) serially diluted in 0.1% peptone water were surface plated on DRBC, APDA, and DG18. All plates were incubated upright in the dark at 25°C. Yeast and mold colonies (15 to 150) formed on RYM Petrifilm plates, YM Petrifilm plates, DRBC, APDA, and DG18 within 48 ± 2, 72 ± 2, and 120 ± 2 h were counted. The same plates were examined by the same person after each consecutive incubation time. In addition, a separate set of DRBC, APDA, and DG18 plates was prepared and examined only after incubating for 120 h.

Statistical analysis. Values for yeast, mold, and yeast plus mold counts for each test parameter combination were analyzed with a general linear model using SAS software (version 9.1, SAS Institute, Cary, NC). A value of 1 CFU/g or ml (0 log CFU/g or ml) was used when no yeasts and/or molds were detected (<10 CFU/g

TABLE 2. *Yeast populations determined by plating foods and food ingredients on mycological enumeration media incubated for 48, 72, and 120 h at 25°C*

Product	Incubation time (h)	Yeast population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Dairy products						
Cheese (Cheddar)	48	A 7.11 a	B <1.00 b	A 7.10 a	A 7.11 a	A 7.14 a
	72	A 7.11 a	A 7.20 a	A 7.10 a	A 7.11 a	A 7.14 a
	120	A 7.11 a	A 7.20 a	A 7.10 a	A 7.11 a	A 7.14 a
Cheese (Gouda)	48	B <1.00 b	B <1.00 b	A 6.36 a	A 6.30 a	A 6.28 a
	72	A 6.27 a	B <1.00 b	A 6.36 a	A 6.30 a	A 6.29 a
	120	A 6.27 a	A 6.12 a	A 6.36 a	A 6.30 a	A 6.29 a
Cheese (Swiss)	48	B <1.00 c	B <1.00 c	A 4.17 a	A 4.00 b	A 3.93 b
	72	A 3.97 b	A 4.02 b	A 4.19 a	A 4.02 b	A 3.96 b
	120	A 3.99 bc	A 4.05 b	A 4.19 a	A 4.03 bc	A 3.97 c
Yogurt (black cherry)	48	A 4.76 ab	B TSTC c	A 4.82 a	A 4.74 b	B TSTC c
	72	A 4.76 b	A 4.93 a	A 4.82 ab	A 4.74 b	A 4.75 b
	120	A 4.76 b	A 4.93 a	A 4.82 ab	A 4.74 b	A 4.75 b
Yogurt (peach)	48	B 3.06 c	B TSTC d	A 3.51 b	B 4.06 a	B TSTC d
	72	A 4.10 b	A 4.14 b	A 3.72 c	A 4.20 a	B TSTC d
	120	A 4.10 b	A 4.18 ab	A 3.72 d	A 4.20 a	A 4.00 c
Meat and meat products						
Ham	48	A 7.69 bc	B <1.00 c	A 8.07 ab	A 8.17 a	A 8.00 ab
	72	A 8.02 a	A 8.05 a	A 8.07 a	A 8.17 a	A 8.01 a
	120	A 8.02 a	A 8.06 a	A 8.07 a	A 8.17 a	A 8.01 a
Salami (summer)	48	B <1.00 b	C <1.00 b	B 4.23 a	A 4.20 a	A 4.22 a
	72	A 4.01 b	B 4.00 b	AB 4.25 a	A 4.21 a	A 4.22 a
	120	A 4.03 b	A 4.05 b	A 4.26 a	A 4.21 a	A 4.22 a
Sausage (ground)	48	A 4.92 ab	B <1.00 c	A 4.93 ab	A 4.79 b	A 5.04 a
	72	A 4.93 a	A 5.06 a	A 4.97 a	A 4.84 a	A 5.05 a
	120	A 4.99 a	A 5.06 a	A 4.97 a	A 4.84 a	A 5.05 a

TABLE 2. *Continued*

Product	Incubation time (h)	Yeast population (log CFU/g or ml)[a] RYM	YM	DRBC	APDA	DG18
Fruits and fruit products						
Apples (sliced)	48	A 6.07 a	B <1.00 b	A 6.08 a	A 6.05 a	B <1.00 b
	72	A 6.07 b	B <1.00 c	A 6.08 b	A 6.05 b	A 6.26 a
	120	A 6.07 b	A 6.13 b	A 6.08 b	A 6.05 b	A 6.26 a
Banana chips (dried)	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	120	A 5.56 a	A 5.65 a	A 4.63 b	A 4.75 b	A 4.88 b
Fruit punch conc (frozen)	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 1.99 a	A 1.99 a	A 1.73 a	A 1.97 a	A 2.07 a
	120	A 2.01 a	A 2.05 a	A 1.90 a	A 1.99 a	A 2.14 a
Grapefruit	48	A <1.00 b	A <1.00 b	A 2.30 a	A 2.00 ab	B <1.00 b
	72	A <1.00 b	A <1.00 b	A 2.30 b	A 2.00 b	A 2.70 a
	120	A <1.00 b	A <1.00 b	A 2.30 b	A 2.00 b	A 2.70 a
Grapefruit juice conc (frozen)	48	B <1.00 a	C <1.00 a	A <1.00 a	A <1.00 a	B <1.00 a
	72	A 1.37 ab	B 1.00 bc	A <1.00 c	A 1.30 ab	A 1.48 a
	120	A 1.43 a	A 1.64 a	A 1.14 a	A 1.43 a	A 1.52 a
Lemon juice	48	B 4.00 a	B <1.00 b	A 4.12 a	A 4.01 a	A 3.87 a
	72	A 4.17 a	A 4.19 a	A 4.14 a	A 4.03 a	A 4.00 a
	120	A 4.17 a	A 4.19 a	A 4.14 a	A 4.03 a	A 4.02 a
Orange	48	B <1.00 c	B <1.00 c	A 4.76 b	A 4.86 a	A 4.80 b
	72	A 4.69 c	B <1.00 d	A 4.76 b	A 4.87 a	A 4.82 a
	120	A 4.69 c	A 4.61 c	A 4.77 b	A 4.87 a	A 4.83 a
Orange-carrot juice	48	B 4.23 b	C 4.15 c	A 4.31 a	A 4.32 a	A 4.31 a
	72	A 4.33 a	B 4.24 b	A 4.33 a	A 4.37 a	A 4.37 a
	120	A 4.33 a	A 4.29 a	A 4.34 a	A 4.38 a	A 4.38 a
Pear-apple juice	48	A 4.62 a	A 4.76 a	A 4.61 a	A 4.71 a	A 4.69 a
	72	A 4.64 a	A 4.76 a	A 4.61 a	A 4.72 a	A 4.70 a
	120	A 4.64 a	A 4.76 a	A 4.62 a	A 4.72 a	A 4.70 a
Raspberries	48	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a
	72	A <1.00 a	A <1.00 a	A 1.24 a	A 0.87 a	A <1.00 a
	120	A <1.00 a	A <1.00 a	A 1.32 a	A 0.87 a	A <1.00 a
Strawberries	48	B <1.00 b	B <1.00 b	A 3.54 a	A 3.54 a	B <1.00 b
	72	A 3.34 bc	A 3.00 c	A 3.56 a	A 3.60 a	A 3.46 ab
	120	A 3.34 bc	A 3.00 c	A 3.56 a	A 3.60 a	A 3.46 ab
Vegetables						
Broccoli	48	B 4.19 c	C 4.05 d	A 4.42 a	A 4.34 b	B TSTC e
	72	A 4.35 b	B 4.17 c	A 4.45 a	A 4.37 b	B TSTC d
	120	A 4.35 b	A 4.28 b	A 4.45 a	A 4.37 b	A 4.31 b
Lettuce (leaf)	48	A 3.52 a	B 3.20 b	A 3.65 a	A 3.61 a	B TSTC b
	72	A 3.63 a	A 3.59 a	A 3.71 a	A 3.66 a	B TSTC b
	120	A 3.64 a	A 3.59 a	A 3.72 a	A 3.67 a	A 3.62 a
Tomato	48	B <1.00 a	B <1.00 a	A 2.52 a	A 2.75 a	A 1.83 a
	72	A 2.75 a	A 2.92 a	A 2.70 a	A 3.08 a	A 2.73 a
	120	A 2.85 a	A 2.92 a	A 2.73 a	A 3.08 a	A 2.60 a
Cereal products						
Bread (walnut, raisin, cinnamon)	48	B <1.00 b	B <1.00 b	A 8.43 a	A 8.55 a	B <1.00 b
	72	A 8.60 a	A 8.76 a	A 8.45 a	A 8.55 a	A 8.46 a
	120	A 8.60 a	A 8.76 a	A 8.45 a	A 8.56 a	A 8.48 a
Bread dough (frozen)	48	A 8.42 a	A 8.39 ab	A 8.29 bc	A 8.30 bc	A 8.17 c
	72	A 8.42 a	A 8.39 ab	A 8.29 bc	A 8.30 bc	A 8.22 c
	120	A 8.42 a	A 8.40 ab	A 8.30 bc	A 8.30 bc	A 8.24 c
Corn flour	48	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a
	72	A <1.00 a	A <1.00 a	A 1.38 a	A 1.14 a	A <1.00 a
	120	A <1.00 a	A <1.00 a	A 1.38 a	A 1.14 a	A 0.85 a
Ground wheat	48	A <1.00 b	A <1.00 b	B <1.00 b	A 1.01 a	A <1.00 b
	72	A 1.24 b	A <1.00 b	A 2.04 a	A 1.31 b	A <1.00 b
	120	A 1.24 b	A <1.00 b	A 2.17 a	A 1.37 b	A 1.31 b

TABLE 2. *Continued*

Product	Incubation time (h)	Yeast population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Seeds and nuts						
Lentils	48	B <1.00 b	B <1.00 b	C 1.78 ab	C 2.08 a	C 0.60 b
	72	B 0.60 c	B <1.00 c	B 2.52 ab	B 2.60 a	B 2.41 b
	120	A 2.60 a	A 2.73 a	A 2.73 a	A 2.79 a	A 2.78 a
Quinoa seeds	48	A 2.59 b	A 2.59 b	B 2.61 b	B 2.69 a	B <1.00 c
	72	A 2.65 b	A 2.67 ab	B 2.65 b	B 2.72 a	B 2.55 c
	120	A 3.78 a	A 3.73 a	A 3.54 a	A 3.32 a	A 3.71 a
Walnuts (raw halves)	48	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a	A <1.00 a
	72	A <1.00 b	A <1.00 b	A 1.23 a	A 0.85 ab	A 0.60 ab
	120	A <1.00 a	A <1.00 a	A 1.23 a	A 0.85 a	A 1.01 a
Seasonings (dry) and tea						
Onion (blended mix)	48	A <1.00 a	A <1.00 a	A 2.10 a	A 2.07 a	A 0.60 a
	72	A <1.00 a	A <1.00 a	A 2.20 a	A 2.09 a	A 0.85 a
	120	A <1.00 a	A <1.00 a	A 2.20 a	A 2.09 a	A 1.01 a
Parsley	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 c	B <1.00 c	A 1.70 b	A 1.99 a	AB 1.60 b
	120	A 1.88 a	A 1.43 a	A 1.82 a	A 2.03 a	A 2.08 a
Pepper (green jalapeno)	48	C <1.00 c	A <1.00 c	A 2.05 b	A 2.30 a	C 0.60 c
	72	B 2.01 b	A 1.60 b	A 2.09 ab	A 2.36 a	B 1.87 b
	120	A 2.26 a	A 2.11 a	A 2.09 a	A 2.38 a	A 2.04 a
Pepper (jalapeno)	48	B <1.00 b	B <1.00 b	A 3.55 a	A 3.56 a	B <1.00 b
	72	A 3.53 a	A 3.19 b	A 3.60 a	A 3.58 a	A 3.53 a
	120	A 3.54 a	A 3.41 a	A 3.62 a	A 3.59 a	A 3.55 a
Tea (black)	48	A <1.00 a	A <1.00 a	A 1.03 a	A 1.37 a	B <1.00 a
	72	A <1.00 a	A <1.00 a	A 1.24 a	A 1.37 a	B 0.60 a
	120	A<1.00 a	A <1.00 a	A 1.24 a	A 1.37 a	A 1.12 a

[a] Fifty-six foods and food ingredients were plated on rapid yeast and mold Petrifilm (RYM), yeast and mold Petrifilm (YM), dichloran rose bengal chloramphenicol agar (DRBC), acidified potato dextrose agar (APDA), and dichloran 18% glycerol agar (DG18). Yeasts were detected in 34 products. Within a row, values not followed by the same lowercase letter are significantly different ($P \leq 0.05$). Within each product and column, values not preceded by the same uppercase letter are significantly different ($P \leq 0.05$). The limit of detection was 1 log CFU/g or ml. Values <1 log CFU/g or ml indicate that yeasts were detected in one or two of the three replicate samples. TSTC, too small to count.

or ml). The least significant difference test was used to determine the significance of differences in mean values ($P \leq 0.05$). Standard linear regression analyses were used to calculate correlation coefficients, slopes, and intercepts. Coefficients of variation were calculated by dividing the repeatability standard deviation by the mean count and expressing this value as a percentage.

RESULTS AND DISCUSSION

Of the 56 foods and food ingredients analyzed for yeast and mold levels, 3 (5.4%) products were marketed frozen, 19 (33.9%) were refrigerated, and 34 (60.7%) were generally stored at ambient temperatures. Twenty-seven products (48.2%) with $a_w > 0.70$ were marketed and are stored either frozen or refrigerated, and five products (8.9%) with $a_w > 0.70$ are stored at ambient temperature. The remaining 24 products (42.9%) had a_w values of <0.70 and are stored at ambient temperature. Products were divided into seven groups: 6 dairy products, 5 meat and meat products, 15 fruits and fruit products, 5 vegetables, 9 cereal products, 8 seeds and nuts, and 8 dry seasonings and tea products.

The presence and number of specific genera and species of yeasts and molds naturally present in various types of spoiled and unspoiled foods and food ingredients has been described *(5, 17, 18)*. The prevalence of either field or storage molds on cereals, legumes, and nuts and spoilage of distinct food types (e.g., citrus fruits, cheeses, and intermediate-a_w foods) by certain yeast or mold genera are also well documented *(5, 27)*. The wide range in composition, pH, a_w, and degree of processing of foods and food ingredients examined in our study would be predicted to result in a diverse range of naturally occurring yeast and mold contaminants. A portion of the mycobiota would likely be in a debilitated physiological state and, unlike healthy cells cultured on laboratory media, would require near-optimum conditions to resuscitate and form colonies on enumeration media. By analyzing foods naturally contaminated with a wide range of fungal species rather than foods artificially inoculated with a limited number of healthy, laboratory-cultured yeasts and molds that may or may not be commonly found in these foods, a more realistic comparison of the suitability of mycological

TABLE 3. *Number and percentage of foods and food ingredients negative for yeasts, molds, and yeasts plus molds when samples were plated on five enumeration media and incubated for 48, 72, and 120 h at 25°C*

No. of foods or ingredients[a]	Microorganisms	Incubation time (h)	No. (%) of samples negative (<1 log CFU/g or ml) for colony formation[b]				
			RYM	YM	DRBC	APDA	DG18
34	Yeasts	48	21 (61.8)	26 (76.5)	8 (23.6)	7 (20.6)	15 (44.1)
		72	8 (23.5)	13 (38.2)	2 (5.9)	1 (2.9)	4 (11.8)
		120	6 (17.6)	7 (20.6)	0 (0)	0 (0)	1 (2.9)
43	Molds	48	29 (67.4)	32 (74.4)	11 (25.6)	11 (25.6)	36 (83.7)
		72	6 (14.0)	7 (16.3)	2 (4.6)	2 (4.6)	8 (18.6)
		120	2 (4.6)	2 (4.6)	0 (0)	0 (0)	0 (0)
56	Yeasts + molds	48	31 (55.4)	41 (73.2)	12 (21.4)	11 (19.6)	40 (71.4)
		72	6 (10.7)	8 (14.3)	2 (3.6)	1 (1.8)	11 (19.6)
		120	2 (3.6)	1 (1.8)	0 (0)	0 (0)	0 (0)

[a] Yeasts, molds, and yeasts plus molds were detected in or on 34, 43, and 56, respectively. For foods and food ingredients in which no yeasts or molds were detected (<1 log CFU/g or ml), counts for yeasts plus molds reflect only mold populations or only yeast populations, respectively.

[b] Values indicate the number (percentage) of samples out of 34 (yeasts), 43 (molds), or 56 (yeasts plus molds) foods and food ingredients in which no yeasts, molds, or yeasts plus molds, respectively, were detected.

enumeration media to support colony formation can be obtained.

Mycological counts were obtained on DRBC, APDA, and DG18 plates incubated for 48, 72, and 120 h and on a second set of these plates incubated for 120 h without handling at 48 and 72 h. This second set was evaluated to determine whether physical handling of plates at 48 and 72 h may have caused dispersal of spores or conidia and development of satellite colonies at 120 h, thereby resulting in inaccurate counts of fungal propagules originally present in the test samples. Results are shown in Table 1. Of the 56 products examined 34, 43, and 56 contained yeasts, molds, or both yeasts and molds, respectively. Of the 34 foods and food ingredients containing yeasts ($\geq$10 CFU/g or ml), significantly higher colony counts ($P \leq 0.05$) were obtained for 10 products plated on DRBC, APDA, and DG18 plates that had been handled at 48 and 72 h before final counts were made at 120 h compared with counts from plates incubated for 120 h without handling at 48 and 72 h; 8 products had significantly lower counts ($P \leq 0.05$). A similar trend was observed for mold counts and total yeast and mold counts, indicating possible dispersal of conidia or spores on plates handled at 48 or 72 h. Disparate values using the two counting procedures were most pronounced on APDA. Considering that the total counts were 102, 129, and 168 for yeasts, molds, and yeasts plus molds, respectively, on the three media, 9.8, 10, and 11.3%, respectively, were significantly higher on plates handled at 48 and 72 h before final counts were made at 120 h compared with counts on plates not handled before counting at 120 h; 7.8, 3.9, and 5.4% of the counts, respectively, were significantly lower. We concluded that, on balance, handling plates at 48 and 72 h did not result in a substantial increase in counts at 120 h. Counts from plates not handled at 48 and 72 h were not considered in further evaluations of the five enumeration media.

Foods and food ingredients analyzed in this study were characterized by diverse origin (plant and animal), composition, pH, and a_w. Pre- and postprocessing treatments, types of packaging, and storage conditions also differed among the test products. These factors greatly enhanced the likelihood that numerous genera and species of naturally occurring yeasts and molds were present on or in the products at the time of analysis. Among the contaminants, xerophilic and nonxerophilic species and propagules in various states of physiological or structural injury caused by exposure to environmental stresses would be expected to be present. Some of the test products produced visual growth of yeasts or molds, resulting in high counts, whereas other products had no evidence of growth.

Yeast counts obtained on 34 of the 56 foods and food ingredients plated on the five test media and incubated for 48, 72, and 120 h at 25°C are listed in Table 2. Molds at lower levels may also have been present in some of these products, but the high dilutions necessary to enable yeast colonies to be counted (50 to 150 per plate) resulted in our inability to detect them. Detection (visualization) of yeast colonies on media incubated for 48 h was, in many cases, not achieved. Of 34 foods and food ingredients, yeast colonies were not visible at 48 h in 21 (61.8%), 26 (76.5%), 8 (23.5%), 7 (20.6%), and 15 (44.1%) of the samples plated on RYM Petrifilm, YM Petrifilm, DRBC, APDA, and DG18, respectively (Table 3). These numbers decreased to 8, 13, 2, 1, and 4 samples, respectively, at 72 h and 6, 7, 0, 0, and 1 sample, respectively, at 120 h. Regardless of the incubation time, DRBC and APDA were the best media for supporting colony development by yeasts present in the 34 test products.

Counts for molds detected in 43 of the 56 foods and food ingredients plated on the five test media and incubated for 48, 72, and 120 h at 25°C are shown in Table 4. Although some of these products also contained yeasts (Table 2), other products (e.g., cereal products, seeds, and nuts), which are more likely to contain molds, appear in this list. This result does not mean that yeasts were absent but rather that populations were not high enough to be detected in diluted samples. As observed

TABLE 4. *Mold populations determined by plating foods and food ingredients on mycological enumeration media incubated for 48, 72, and 120 h at 25°C*

Product	Incubation time (h)	Mold population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Dairy products						
Cheese (Cheddar)	48	C <1.00 a	B <1.00 a	C <1.00 a	B <1.00 a	B <1.00 a
	72	B 4.67 ab	A 4.92 a	B 4.52 bc	A 4.88 a	A 4.00 c
	120	A 4.95 a	A 4.94 a	A 4.85 a	A 4.92 a	A 4.85 a
Cheese (Gouda)	48	B <1.00 b	B <1.00 b	A 5.57 a	A 5.61 a	B <1.00 b
	72	A 5.61 a	A 5.71 a	A 5.57 a	A 5.61 a	A 5.54 a
	120	A 5.61 a	A 5.71 a	A 5.57 a	A 5.61 a	A 5.54 a
Yogurt (blueberry)	48	A 2.77 b	B 2.70 b	A 2.89 a	A 2.77 b	B TSTC c
	72	A 2.81 b	A 2.88 ab	A 2.93 a	A 2.82 b	B TSTC c
	120	A 2.81 bc	A 2.90 ab	A 2.93 a	A 2.82 bc	A 2.77 c
Meat and meat products						
Beef jerky	48	A <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 6.44 ab	A 6.46 ab	A 6.59 a	A 6.60 a	A 5.73 b
	120	A 6.45 ab	A 6.46 ab	A 6.60 a	A 6.61 a	A 5.73 b
Chicken salad	48	A 2.48 a	A <1.00 b	A 2.65 a	B 2.66 a	B <1.00 b
	72	A 2.71 a	A 2.77 a	A 2.77 a	A 2.79 a	A 2.68 a
	120	A 2.71 a	A 2.78 a	A 2.79 a	A 2.81 a	A 2.75 a
Fruits and fruit products						
Banana chips (dried)	48	B <1.00 a	B <1.00 a	A <1.00 a	A <1.00 a	B <1.00 a
	72	B <1.00 a	B <1.00 a	A <1.00 a	B <1.00 a	B <1.00 a
	120	A 5.50 b	A 5.19 b	A 3.75 b	A 5.63 b	A 6.02 a
Date paste	48	B <1.00 a	B <1.00 a	B 1.30 a	B 0.60 a	B <1.00 a
	72	AB 1.56 c	AB 1.60 bc	A 2.04 a	A 1.88 ab	B 1.48 c
	120	A 2.05 a	A 2.17 a	A 2.14 a	A 2.01 a	A 2.08 a
Grapefruit	48	B 3.40 c	C <1.00 c	A 4.20 a	A 3.99 b	B <1.00 c
	72	A 3.68 c	B 3.29 c	A 4.22 a	A 4.01 b	A 3.65 c
	120	A 3.68 c	A 3.36 c	A 4.22 a	A 4.01 b	A 3.66 c
Orange	48	B <1.00 c	B <1.00 c	A 4.87 a	B 4.63 b	B 3.88 c
	72	A 4.56 c	B <1.00 d	A 4.91 a	A 4.75 b	A 4.66 c
	120	A 4.59 c	A 4.67 c	A 4.92 a	A 4.75 b	A 4.66 c
Pear-apple juice	48	B <1.00 a	A <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 a	A <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	120	A 2.99 a	A 2.37 c	A 2.73 bc	A 2.43 c	A 2.92 ab
Pineapple (dried)	48	A <1.00 b	A <1.00 b	A 0.60 b	A 1.12 a	A <1.00 b
	72	A <1.00 b	A <1.00 b	A 0.60 b	A 1.22 a	A <1.00 b
	120	A <1.00 b	A <1.00 b	A 1.30 ab	A 1.37 a	A 0.85 ab
Raisins 1	48	B <1.00 b	C <1.00 b	A 1.75 a	B 1.48 ab	C <1.00 b
	72	A 1.90 a	B 1.85 a	A 2.08 a	A 2.04 a	B 1.88 a
	120	A 1.95 b	A 1.88 b	A 2.18 a	A 2.08 ab	A 2.19 a
Raisins 2	48	C <1.00 b	C <1.00 b	B 1.64 a	B 1.37 ab	B <1.00 b
	72	B 1.70 ab	B 1.67 ab	AB 1.92 a	A 1.88 a	B 1.43 b
	120	A 1.80 c	A 1.82 c	A 2.17 ab	A 1.99 bc	A 2.18 a
Raspberries	48	A 3.14 a	B 3.13 a	A 3.22 a	A 3.17 a	B TSTC b
	72	A 3.19 a	A 3.18 a	A 3.27 a	A 3.22 a	A 3.18 a
	120	A 3.20 a	A 3.18 a	A 3.28 a	A 3.24 a	A 3.22 a
Strawberries	48	A 4.01 a	A 4.00 a	A 4.07 a	A 4.07 a	B <1.00 b
	72	A 4.02 a	A 4.02 a	A 4.09 a	A 4.09 a	A 4.09 a
	120	A 4.02 a	A 4.02 a	A 4.09 a	A 4.17 a	A 4.10 a
Vegetables						
Broccoli	48	B 2.78 a	A 2.78 a	A 2.75 a	A 2.70 a	B TSTC b
	72	A 3.04 a	A 3.05 a	A 2.82 a	A 2.78 a	B TSTC b
	120	A 3.04 a	A 3.05 a	A 2.82 a	A 2.85 a	A 3.04 a
Lettuce (leaf)	48	A 3.11 b	B 2.94 b	A 3.32 a	A 3.29 a	B TSTC c
	72	A 3.19 b	A 3.09 b	A 3.39 a	A 3.40 a	B TSTC c
	120	A 3.23 ab	A 3.12 b	A 3.39 a	A 3.40 a	A 3.31 ab

TABLE 4. *Continued*

Product	Incubation time (h)	Mold population (log CFU/g or ml)[a] RYM	YM	DRBC	APDA	DG18
Onions (green)	48	B <1.00 a	A <1.00 a	C <1.00 a	B <1.00 a	B <1.00 a
	72	B 0.60 b	A 1.03 b	B 1.56 a	B 1.52 a	B <1.00 b
	120	A 1.00 b	A 1.23 b	A 1.75 a	A 1.67 a	A 1.22 b
Potato flakes	48	B 1.22 b	B <1.00 c	A 1.70 a	B 1.30 b	C <1.00 c
	72	A 1.75 ab	B 1.23 c	A 1.85 a	B 1.52 abc	B 1.70 abc
	120	A 1.87 ab	A 1.64 b	A 1.97 a	A 2.03 a	A 2.01 a
Tomato	48	A 3.67 b	A 3.57 b	A 3.85 a	A 3.73 ab	A 3.70 b
	72	A 3.71 bc	A 3.60 c	A 3.89 a	A 3.79 ab	A 3.76 b
	120	A 3.72 bc	A 3.64 c	A 3.89 a	A 3.79 b	A 3.76 bc
Cereal products						
Bread (oatmeal)	48	B <1.00 c	B <1.00 c	A 8.26 b	A 8.37 a	B <1.00 c
	72	A 8.18 b	A 7.97 c	A 8.28 ab	A 8.39 a	A 8.23 b
	120	A 8.19 bc	A 8.01 c	A 8.28 ab	A 8.39 a	A 8.23 b
Bread (walnut, raisin, cinnamon)	48	A <1.00 a	A <1.00 a	A 6.00 a	A 5.82 a	A <1.00 a
	72	A 6.22 a	A 6.22 a	A 6.00 a	A 6.00 a	A 6.00 a
	120	A 6.30 a	A 6.22 a	A 6.00 a	A 6.12 a	A 6.00 a
Bread crumbs (seasoned)	48	B <1.00 b	B <1.00 b	B 1.64 a	B 1.14 ab	B 0.87 b
	72	A 2.37 b	A 2.33 b	A 2.59 a	A 2.49 ab	B 1.12 c
	120	A 2.41 b	A 2.37 b	A 2.67 a	A 2.54 ab	A 2.52 ab
Corn flour	48	C <1.00 b	B <1.00 b	C 1.70 a	C 1.67 a	A <1.00 b
	72	B 1.88 c	AB 1.80 cd	B 2.16 b	B 2.32 a	A 1.30 d
	120	A 2.08 bc	A 2.14 bc	A 2.38 ab	A 2.51 a	A 1.90 c
Ground corn	48	B <1.00 a	C <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 1.67 b	B 1.60 b	A 1.52 b	A 1.85 a	B <1.00 c
	120	A 1.67 b	A 1.73 b	A 1.73 b	A 1.97 a	A 1.88 ab
Ground wheat	48	C <1.00 b	A <1.00 b	B <1.00 b	B 1.48 a	B <1.00 b
	72	B 1.85 bc	A 1.64 cd	A 2.15 a	A 2.08 ab	B 1.01 d
	120	A 1.97 ab	A 1.82 b	A 2.18 a	A 2.14 a	A 1.82 b
Steel-cut oats	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B 1.12 a	B 1.12 a	B 0.85 a	B <1.23 a	B <1.00 a
	120	A 1.82 a	A 1.82 a	A 1.52 a	A 1.82 a	A 1.73 a
Wheat flour	48	C <1.00 a	C <1.00 a	A 2.12 a	A 2.00 a	A <1.00 a
	72	B 2.36 a	B 2.30 a	A 2.41 a	A 2.36 a	A 1.80 a
	120	A 2.73 a	A 2.73 a	A 2.70 a	A 2.49 a	A 2.67 a
Seeds and nuts						
Chia seeds	48	A <1.00 b	A <1.00 b	B 3.66 a	A 3.51 a	C <1.00 b
	72	A 3.21 b	A 2.88 b	B 3.79 a	A 3.72 a	B 3.62 a
	120	A 3.43 b	A 3.11 b	A 4.04 a	A 3.87 a	A 3.85 a
Hazelnuts (raw)	48	A <1.00 a	B <1.00 a	A <1.00 a	B <1.00 a	B <1.00 a
	72	A 0.87 a	B <1.00 a	A 1.37 a	B 0.85 a	B 1.01 a
	120	A 1.30 a	A 1.22 a	A 1.56 a	A 1.67 a	A 1.48 a
Lentils	48	C <1.00 b	B <1.00 b	A 1.37 a	B <1.00 b	B <1.00 b
	72	B 1.22 b	AB 1.64 ab	A 1.85 a	A 1.43 b	AB 1.31 b
	120	A 2.00 a	A 1.85 abc	A 1.95 ab	A 1.48 b	A 1.67 bc
Peanuts (raw)	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 b	B <1.00 b	A 4.25 a	A 4.26 a	B <1.00 b
	120	A 4.24 a	A 4.12 b	A 4.30 a	A 4.27 a	A 4.30 a
Quinoa seeds	48	A <1.00 a	A <1.00 a	A 0.85 a	A 0.60 a	A <1.00 a
	72	A 1.37 a	A 1.01 a	A 1.12 a	A 1.14 a	A 1.14 a
	120	A 1.37 a	A 1.01 a	A 1.12 a	A 1.14 a	A 1.14 a
Sunflower seeds	48	B 1.30 a	B 1.01 a	A 1.56 a	A 1.31 a	B <1.00 a
	72	A 1.78 a	B 1.56 a	A 1.70 a	A 1.73 a	B 1.60 a
	120	A 1.85 b	A 1.85 b	A 1.87 b	A 1.80 b	A 2.12 a
Walnuts (raw halves)	48	B <1.00 a	B <1.00 a	B 1.01 a	B <1.00 a	C <1.00 a
	72	AB 1.56 a	B 1.01 a	AB 1.85 a	B 1.60 a	B 1.67 a
	120	A 1.70 b	A 1.73 b	A 1.95 b	A 1.75 b	A 2.20 a

TABLE 4. *Continued*

Product	Incubation time (h)	Mold population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Walnuts (raw pieces)	48	A 3.46 bc	B 3.22 c	A 3.67 a	A 3.52 b	B <1.00 d
	72	A 3.52 b	A 3.36 b	A 3.70 a	A 3.55 b	A 3.50 b
	120	A 3.53 b	A 3.37 b	A 3.70 a	A 3.55 b	A 3.51 b
Seasonings (dry) and tea						
Onion (blend)	48	B 0.87 b	C 1.01 b	C 1.48 a	B 1.48 a	B <1.00 b
	72	AB 1.48 bc	B 1.43 bc	B 1.92 a	B 1.75 ab	B <1.00 c
	120	A 1.80 b	A 1.75 b	A 2.17 a	A 2.09 a	A 2.11 a
Onion (blended mix)	48	B 0.60 a	C 1.01 a	C 1.53 a	C 1.23 a	B <1.00 a
	72	B 1.60 b	B 1.87 b	B 2.32 a	B 1.95 b	B 0.60 b
	120	A 2.38 bc	A 2.26 c	A 2.61 a	A 2.46 b	A 2.45 b
Onion (powder)	48	C <1.00 b	C 0.85 b	C 1.78 a	C 1.75 a	B <1.00 b
	72	B 2.37 b	B 2.30 b	B 2.55 a	B 2.53 a	B 1.01 c
	120	A 2.75 a	A 2.67 a	A 2.81 a	A 2.72 a	A 2.63 a
Pepper (green jalapeno)	48	A <1.00 a	B <1.00 a	A 0.60 a	A 0.87 a	A <1.00 a
	72	A <1.00 a	A <1.00 a	B <1.01 a	A 0.87 a	A 1.01 a
	120	A <1.00 b	A <1.00 b	A 1.43 a	A 1.01 b	A 1.14 ab
Pepper (jalapeno)	48	B 2.30 bc	B <1.00 c	A 2.78 a	A 2.52 b	B <1.00 c
	72	A 2.94 a	A 2.87 a	A 2.82 a	A 2.70 a	A 2.85 a
	120	A 2.94 a	A 2.90 a	A 2.67 a	A 2.75 a	A 2.88 a
Tea (black)	48	B <1.00 b	B <1.00 b	A 2.21 a	A 2.12 a	B <1.00 b
	72	A 2.00 b	B 1.83 b	A 2.36 a	A 2.21 ab	A 2.07 b
	120	A 2.07 a	A 2.30 a	A 2.39 a	A 2.25 a	A 2.2 9 a
Tea (green)	48	B <1.00 a	B <1.00 a	B <1.00 a	B 0.60 a	B <1.00 a
	72	B <1.00 a	B 0.60 b	B 1.37 a	B 0.86 b	B 0.85 b
	120	A 1.64 a	A 1.56 a	A 1.70 a	A 1.60 a	A 2.00 a

[a] Fifty-six foods and food ingredients were plated on rapid yeast and mold Petrifilm (RYM), yeast and mold Petrifilm (YM), dichloran rose bengal chloramphenicol agar (DRBC), acidified potato dextrose agar (APDA), and dichloran 18% glycerol agar (DG18). Molds were detected in 43 products. Within a row, values not followed by the same lowercase letter are significantly different ($P \leq 0.05$). Within each product and column, values not preceded by the same uppercase letter are significantly different ($P \leq 0.05$). The limit of detection was 1 log CFU/g or ml. Values <1 log CFU/g or ml indicate that molds were detected in one or two of the three replicate samples. TSTC, too small to count.

TABLE 5. *Yeast and mold populations determined by plating foods and food ingredients on mycological enumeration media incubated for 48, 72, and 120 h at 25°C*

Product	Incubation time (h)	Yeast and mold population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Dairy products						
Cheese (Cheddar)	48	A 7.11 a	B <1.00 b	A 7.10 a	A 7.11 a	A 7.14 a
	72	A 7.12 a	A 7.20 a	A 7.10 a	A 7.12 a	A 7.14 a
	120	A 7.12 a	A 7.20 a	A 7.10 a	A 7.12 a	A 7.14 a
Cheese (Gouda)	48	B <1.00 b	B <1.00 b	A 6.42 a	A 6.38 a	A 6.28 a
	72	A 6.36 a	B 5.71 b	A 6.42 a	A 6.38 a	A 6.36 a
	120	A 6.36 a	A 6.26 a	A 6.42 a	A 6.38 a	A 6.36 a
Cheese (Swiss)	48	B <1.00 c	B <1.00 c	A 4.17 a	A 4.00 b	A 3.93 b
	72	A 3.97 b	A 4.02 b	A 4.19 a	A 4.02 b	A 3.96 b
	120	A 3.99 bc	A 4.05 b	A 4.19 a	A 4.03 bc	A 3.97 c
Yogurt (black cherry)	48	A 4.75 ab	B <1.00 c	A 4.82 a	A 4.74 b	B <1.00 c
	72	A 4.76 b	A 4.93 a	A 4.82 ab	A 4.74 b	A 4.75 b
	120	A 4.76 b	A 4.93 a	A 4.82 ab	A 4.74 b	A 4.75 b
Yogurt (blueberry)	48	A 2.77 b	B 2.70 b	A 2.89 a	A 2.77 b	B <1.00 c
	72	A 2.81 b	A 2.88 ab	A 2.93 a	A 2.82 b	B <1.00 c
	120	A 2.81 bc	A 2.90 ab	A 2.93 a	A 2.82 bc	A 2.77 c
Yogurt (peach)	48	B 3.06 c	B <1.00 d	A 3.51 b	B 4.06 a	B <1.00 d
	72	A 4.10 b	A 4.14 b	A 3.72 c	A 4.20 a	B <1.00 d
	120	A 4.10 b	A 4.18 ab	A 3.72 d	A 4.20 a	A 4.00 c

TABLE 5. *Continued*

Product	Incubation time (h)	Yeast and mold population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Meat and meat products						
Beef jerky	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 6.44 a	A 6.46 a	A 6.59 a	A 6.60 a	A 5.73 a
	120	A 6.45 a	A 6.46 a	A 6.60 a	A 6.61 a	A 5.73 a
Chicken salad	48	A 2.48 a	A <1.00 b	A 2.65 a	A 2.66 a	B <1.00 b
	72	A 2.71 a	A 2.77 a	A 2.77 a	A 2.79 a	A 2.68 a
	120	A 2.71 a	A 2.78 a	A 2.79 a	A 2.81 a	A 2.75 a
Ham	48	A 7.69 bc	B <1.00 c	A 8.07 ab	A 8.17 a	A 8.00 ab
	72	A 8.02 a	A 8.05 a	A 8.07 a	A 8.17 a	A 8.01 a
	120	A 8.02 a	A 8.06 a	A 8.07 a	A 8.17 a	A 8.01 a
Salami (summer)	48	B <1.00 b	C <1.00 b	B 4.23 a	A 4.20 a	A 4.22 a
	72	A 4.01 b	B 4.00 b	AB 4.25 a	A 4.21 a	A 4.22 a
	120	A 4.03 b	A 4.05 b	A 4.26 a	A 4.21 a	A 4.22 a
Sausage (ground)	48	A 4.92 ab	B <1.00 c	A 4.93 ab	A 4.79 b	A 5.04 a
	72	A 4.93 a	A 5.06 a	A 4.97 a	A 4.84 a	A 5.05 a
	120	A 4.99 a	A 5.06 a	A 4.97 a	A 4.84 a	A 5.05 a
Fruits and fruit products						
Apples (sliced)	48	A 6.07 a	B <1.00 b	A 6.08 a	A 6.05 a	B <1.00 b
	72	A 6.07 b	B <1.00 c	A 6.08 b	A 6.05 b	A 6.26 a
	120	A 6.07 b	A 6.13 b	A 6.08 b	A 6.05 b	A 6.26 a
Banana chips (dried)	48	B <1.00 a	B <1.00 a	B <1.00 a	A <1.00 a	B <1.00 a
	72	B <1.00 a	B <1.00 a	B <1.00 a	A <1.00 a	B <1.00 a
	120	A 5.84 ab	A 5.78 b	A 4.68 c	A 5.68 bc	A 6.05 a
Date paste	48	A <1.00 b	B<1.00 b	B 1.30 a	B 0.60 b	B <1.00 b
	72	A 1.56 c	AB 1.60 bc	A 2.04 a	A 1.88 ab	B 1.48 c
	120	A 2.05 a	A 2.17 a	A 2.14 a	A 2.01 a	A 2.08 a
Fruit punch conc (frozen)	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 1.99 a	A 1.99 a	A 1.73 a	A 1.97 a	A 2.07 a
	120	A 2.01 a	A 2.05 a	A 1.90 a	A 1.99 a	A 2.14 a
Grapefruit	48	A 3.40 c	C <1.00 c	A 4.20 a	A 4.00 b	B <1.00 c
	72	A 3.68 c	B 3.29 c	A 4.22 a	A 4.02 b	A 3.70 c
	120	A 3.68 c	A 3.36 c	A 4.22 a	A 4.02 b	A 3.70 c
Grapefruit conc (frozen)	48	A <1.00 a	C <1.00 a	A <1.00 a	A <1.00 a	B <1.00 a
	72	A 1.37 ab	B 1.00 bc	A <1.00 c	A 1.30 ab	A 1.48 a
	120	A 1.43 a	A 1.64 a	A 1.14 a	A 1.43 a	A 1.52 a
Lemon juice	48	B 4.00 a	B <1.00 b	A 4.12 a	A 4.01 a	A 4.01 a
	72	A 4.17 a	A 4.18 a	A 4.14 a	A 4.03 a	A 4.00 a
	120	A 4.17 a	A 4.19 a	A 4.14 a	A 4.03 a	A 4.02 a
Orange	48	B <1.00 d	B <1.00 d	A 5.12 a	A 5.06 b	B 4.85 c
	72	A 4.93 c	B <1.00 d	A 5.14 a	A 5.11 a	A 5.05 b
	120	A 4.95 c	A 4.94 c	A 5.15 a	A 5.11 a	A 5.06 b
Orange-carrot juice	48	B 4.23 b	C 4.15 c	A 4.31 a	A 4.33 a	A 4.31 a
	72	A 4.33 a	B 4.24 b	A 4.33 a	A 4.37 a	A 4.37 a
	120	A 4.33 a	A 4.29 a	A 4.33 a	A 4.38 a	A 4.38 a
Pear-apple juice	48	A 4.62 a	A 4.76 a	A 4.61 a	A 4.71 a	A 4.69 a
	72	A 4.64 a	A 4.76 a	A 4.61 a	A 4.72 a	A 4.70 a
	120	A 4.66 a	A 4.77 a	A 4.62 a	A 4.73 a	A 4.71 a
Pineapple (dried)	48	A <1.00 b	A <1.00 b	A 0.60 b	A 1.12 a	A <1.00 b
	72	A <1.00 b	A <1.00 b	A 0.60 b	A 1.22 a	A <1.00 b
	120	A <1.00 a	A <1.00 a	A 1.30 a	A 1.37 a	A 0.85 a
Raisins 1	48	B <1.00 b	C <1.00 b	A 1.75 a	B 1.48 ab	C <1.00 b
	72	A 1.90 a	B 1.85 a	A 2.08 a	A 2.04 a	B 1.88 a
	120	A 1.95 b	A 1.88 b	A 2.18 a	A 2.08 ab	A 2.19 a
Raisins 2	48	C <1.00 b	C <1.00 b	B 1.64 a	B 1.37 ab	B <1.00 b
	72	B 1.70 ab	B 1.67 ab	AB 1.92 a	A 1.88 a	B 1.43 b
	120	A 1.80 c	A 1.82 c	A 2.17 ab	A 1.99 bc	A 2.18 a

TABLE 5. *Continued*

Product	Incubation time (h)	Yeast and mold population (log CFU/g or ml)[a]				
		RYM	YM	DRBC	APDA	DG18
Raspberries	48	A 3.14 a	B 3.13 a	A 3.22 a	A 3.17 a	B <1.00 b
	72	A 3.19 a	A 3.18 a	A 3.28 a	A 3.23 a	A 3.18 a
	120	A 3.20 a	A 3.18 a	A 3.29 a	A 3.24 a	A 3.22 a
Strawberries	48	B 4.01 b	A 4.00 b	A 4.18 a	A 4.18 a	B <1.00 c
	72	A 4.10 a	A 4.06 a	A 4.20 a	A 4.21 a	A 4.18 a
	120	A 4.10 a	A 4.06 a	A 4.20 a	A 4.27 a	A 4.19 a
Vegetables						
Broccoli	48	B 4.20 c	C 4.07 d	A 4.43 a	A 4.35 b	B <1.00 e
	72	A 4.37 b	B 4.20 c	A 4.46 a	A 4.38 b	B <1.00 d
	120	A 4.37 b	A 4.31 b	A 4.46 a	A 4.38 b	A 4.33 b
Lettuce (leaf)	48	A 3.66 b	B 3.39 c	A 3.82 a	A 3.78 a	B <1.00 d
	72	A 3.76 bc	A 3.71 c	A 3.88 a	A 3.85 ab	B <1.00 d
	120	A 3.78 a	A 3.72 a	A 3.89 a	A 3.86 a	A 3.79 a
Onions (green)	48	B <1.00 a	A <1.00 a	C <1.00 a	B < 1.00 a	B <1.00 a
	72	B 0.60 b	A 1.03 b	B 1.56 a	A 1.52 a	B <1.00 b
	120	A 1.00 b	A 1.23 b	A 1.75 a	A 1.67 a	A 1.22 b
Potato flakes	48	B 1.22 b	B <1.00 c	A 1.70 a	B 1.30 b	C <1.00 c
	72	A 1.75 ab	B 1.23 c	A 1.85 a	B 1.52 bc	B 1.70 abc
	120	A 1.87 ab	A 1.64 b	A 1.97 a	A 2.03 a	A 2.01 a
Tomato	48	A 3.67 bc	A 3.57 c	A 3.87 a	B 3.77 ab	A 3.70 bc
	72	A 3.76 c	A 3.68 c	A 3.91 a	A 3.87 ab	A 3.80 bc
	120	A 3.78 bc	A 3.71 c	A 3.92 a	A 3.87 ab	A 3.79 bc
Cereal products						
Bread (oatmeal)	48	B <1.00 c	B <1.00 c	A 8.26 b	A 8.37 a	B <1.00 c
	72	A 8.18 b	A 7.97 c	A 8.28 ab	A 8.39 a	A 8.23 b
	120	A 8.19 bc	A 8.01 c	A 8.28 ab	A 8.39 a	A 8.23 b
Bread (walnut, raisin, cinnamon)	48	B <1.00 b	A <1.00 b	A 8.43 a	A 8.55 a	B <1.00 b
	72	A 8.60 a	A 8.76 a	A 8.45 a	A 8.55 a	A 8.47 a
	120	A 8.61 a	A 8.76 a	A 8.45 a	A 8.56 a	A 8.48 a
Bread crumbs (seasoned)	48	B <1.00 a	B <1.00 a	B 1.64 a	B 1.14 a	B 0.87 a
	72	A 2.37 b	A 2.33 b	A 2.59 a	A 2.49 ab	B 1.12 c
	120	A 2.41 b	A 2.37 b	A 2.67 a	A 2.54 ab	A 2.52 ab
Bread dough (frozen)	48	A 8.42 a	A 8.39 ab	A 8.29 bc	A 8.30 bc	A 8.17 c
	72	A 8.42 a	A 8.39 ab	A 8.29 bc	A 8.30 bc	A 8.22 c
	120	A 8.42 a	A 8.40 ab	A 8.30 bc	A 8.30 bc	A 8.24 c
Corn flour	48	B <1.00 b	B <1.00 b	B 1.71 a	B 1.68 a	A <1.00 b
	72	B <1.00 b	B <1.00 b	AB 2.22 a	A 2.34 a	A 1.32 b
	120	A 2.08 c	A 2.14 bc	A 2.42 ab	A 2.53 a	A 1.94 c
Ground corn	48	B <1.00 a	C <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	A 1.67 b	B 1.60 b	A 1.52 b	A 1.85 a	B <1.00 c
	120	A 1.67 b	A 1.73 b	A 1.73 b	A 1.97 a	A 1.88 ab
Ground wheat	48	B <1.00 b	A <1.00 b	B <1.00 b	B 1.61 a	B <1.00 b
	72	A 1.94 bc	A 1.65 cd	A 2.40 a	A 2.15 b	B 1.05 d
	120	A 2.04 bc	A 1.83 c	A 2.47 a	A 2.21 b	A 1.94 c
Steel-cut oats	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B 1.12 a	B 1.12 a	B 0.85 a	B 1.23 a	B <1.00 a
	120	A 1.82 a	A 1.82 a	A 1.52 a	A 1.82 a	A 1.73 a
Wheat flour	48	C <1.00 b	C <1.00 b	A 2.12 a	A 2.00 ab	A <1.00 b
	72	B 2.36 a	B 2.30 a	A 2.41 a	A 2.36 a	A 1.80 a
	120	A 2.73 a	A 2.73 a	A 2.70 a	A 2.49 a	A 2.67 a
Seeds and nuts						
Chia seeds	48	A <1.00 b	A <1.00 b	B 3.66 a	A 3.51 a	C <1.00 b
	72	A 3.21 b	A 2.88 b	B 3.79 a	A 3.72 a	B 3.62 a
	120	A 3.43 b	A 3.11 b	A 4.04 a	A 3.87 a	A 3.85 a
Hazelnuts (raw)	48	A <1.00 a	B <1.00 a	A <1.00 a	B <1.00 a	B <1.00 a
	72	A 0.87 a	B <1.00 a	A 1.37 a	B 0.85 a	B 1.01 a
	120	A 1.30 a	A 1.22 a	A 1.56 a	A 1.67 a	A 1.48 a

TABLE 5. *Continued*

Product	Incubation time (h)	Yeast and mold population (log CFU/g or ml)[a] RYM	YM	DRBC	APDA	DG18
Lentils	48	B <1.00 b	B <1.00 b	C 1.92 a	C 2.08 a	C 0.70 b
	72	B 1.32 c	B 1.65 c	B 2.60 a	B 2.63 a	B 2.45 b
	120	A 2.70 a	A 2.78 a	A 2.80 a	A 2.82 a	A 2.82 a
Peanuts (raw)	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 b	B <1.00 b	A 4.25 a	A 4.26 a	B <1.00 b
	120	A 4.24 a	A 4.12 b	A 4.30 a	A 4.27 a	A 4.30 a
Quinoa seeds	48	A 2.60 b	A 2.59 b	B 2.62 b	B 2.69 a	B <1.00 c
	72	A 2.67 b	A 2.68 b	B 2.66 b	B 2.73 a	B 2.57 c
	120	A 3.78 a	A 3.73 a	A 3.55 a	A 3.33 a	A 3.71 a
Sunflower seeds	48	B 1.30 a	B 1.01 a	A 1.56 a	A 1.31 a	B <1.00 a
	72	A 1.78 a	B 1.56 a	A 1.70 a	A 1.73 a	B 1.60 a
	120	A 1.85 a	A 1.85 a	A 1.87 a	A 1.80 a	A 2.12 a
Walnuts (raw halves)	48	A <1.00 a	B <1.00 a	B 1.05 a	B <1.00 a	C <1.00 a
	72	A 1.58 b	B 1.05 b	A 1.94 a	A 1.67 b	B 1.70 ab
	120	A 1.74 c	A 1.71 c	A 2.03 b	A 1.80 c	A 2.23 a
Walnuts (raw pieces)	48	A 3.46 bc	B 3.22 c	A 3.67 a	A 3.52 b	B <1.00 d
	72	A 3.52 b	A 3.36 b	A 3.70 a	A 3.55 b	A 3.50 b
	120	A 3.53 b	A 3.37 b	A 3.70 a	A 3.55 b	A 3.51 b
Seasonings (dry) and tea						
Onion (blend)	48	B 0.87 b	C 1.01 b	C 1.48 a	B 1.48 a	B <1.00 b
	72	AB 1.48 bc	B 1.43 bc	B 1.92 a	B 1.75 ab	B <1.00 c
	120	A 1.80 b	A 1.75 b	A 2.17 a	A 2.09 a	A 2.11 a
Onion (blended mix)	48	B 0.70 a	C 1.05 a	A 2.21 a	A 2.13 a	B 0.70 a
	72	B 1.61 a	B 1.87 a	A 2.56 a	A 2.33 a	B 1.04 a
	120	A 2.38 b	A 2.26 b	A 2.76 a	A 2.61 ab	A 2.46 b
Onion (powder)	48	C <1.00 b	C 0.85 b	C 1.78 a	C 1.75 a	B <1.00 b
	72	B 2.37 b	B 2.30 b	B 2.55 a	B 2.53 a	B 1.01 c
	120	A 2.75 a	A 2.67 a	A 2.81 a	A 2.72 a	A 2.63 a
Parsley	48	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a	B <1.00 a
	72	B <1.00 c	B <1.00 c	A 1.70 b	A 1.99 a	AB 1.60 b
	120	A 1.88 a	A 1.43 a	A 1.82 a	A 2.02 a	A 2.08 a
Pepper (jalapeno)	48	B 2.30 b	B <1.00 b	A 3.62 a	A 3.60 a	B <1.00 b
	72	A 3.63 a	A 3.36 b	A 3.67 a	A 3.63 a	A 3.61 a
	120	A 3.63 a	A 3.53 a	A 3.67 a	A 3.65 a	A 3.63 a
Pepper (green jalapeno)	48	C <1.00 c	A <1.00 c	A 2.07 b	A 2.32 a	B 0.70 c
	72	B 2.02 a	A 1.61 a	A 2.13 a	A 2.38 a	A 1.92 a
	120	A 2.27 a	A 2.15 a	A 2.18 a	A 2.40 a	A 2.09 a
Tea (black)	48	B <1.00 b	B <1.00 b	A 2.24 a	A 2.25 a	C <1.00 b
	72	A 2.00 bc	B 1.83 c	A 2.39 a	A 2.27 ab	B 2.08 bc
	120	A 2.07 a	A 2.30 a	A 2.42 a	A 2.30 a	A 2.32 a
Tea (green)	48	B <1.00 a	B <1.00 a	B <1.00 a	B 0.60 a	B <1.00 a
	72	B <1.00 b	B 0.60 b	B 1.37 a	B 0.85 b	B 0.85 b
	120	A 1.64 a	A 1.56 a	A 1.70 a	A 1.60 a	A 2.00 a

[a] Fifty-six foods and food ingredients were plated on rapid yeast and mold Petrifilm (RYM), yeast and mold Petrifilm (YM), dichloran rose bengal chloramphenicol agar (DRBC), acidified potato dextrose agar (APDA), and dichloran 18% glycerol agar (DG18). Yeasts, molds, or both yeasts and molds were detected on at least one medium incubated for 120 h. Within a row, values not followed by the same lowercase letter are significantly different ($P \leq 0.05$). Within each product and column, values not preceded by the same uppercase letter are significantly different ($P \leq 0.05$). The limit of detection was 1 log CFU/g or ml. Values <1 log CFU/g or ml indicate that yeasts and/or molds were detected in one or two of the three replicate samples.

with yeast counts, in many cases, mold colonies were not detected on media incubated for only 48 h. Of the 43 foods and food ingredients analyzed, mold colonies were not visible at 48 h in 29 (67.4%), 32 (74.4%), 11 (25.6%), 11 (25.6%), and 36 (83.7%) samples plated on RYM Petrifilm, YM Petrifilm, DRBC, APDA, and DG18, respectively (Table 3). These numbers decreased to 6, 7, 2, 2, and 8 samples, respectively, at 72 h and 2, 2, 0, 0, and 0 samples, respectively, at 120 h. As with yeast counts, regardless of the incubation time, mold counts were generally higher on DRBC and APDA than on other media. Colony formation by yeasts and molds in low-a_w foods would be expected to be slow, requiring more than 48 h for visual detection. Although some counts would

TABLE 6. *Statistical comparisons of yeast, mold, and yeast plus mold populations recovered from 56 foods and food ingredients on mycological enumeration media incubated for 48, 72, and 120 h at 25°C*

Measure of performance	Microorganisms	Incubation time (h)	Value difference									
			RYM vs:				YM vs:			DRBC vs:		APDA vs DG18
			YM	DRBC	APDA	DG18	DRBC	APDA	DG18	APDA	DG18	
Correlation coefficient (r^2)	Yeasts	48	0.34	0.35	0.34	0.23	0.11	0.11	0.11	0.97	0.46	0.44
		72	0.66	0.91	0.93	0.73	0.59	0.61	0.42	0.97	0.77	0.78
		120	0.99	0.92	0.95	0.93	0.92	0.95	0.93	0.97	0.93	0.96
	Molds	48	0.70	0.18	0.17	0.03	0.12	0.12	0.05	0.93	0.08	0.07
		72	0.83	0.72	0.81	0.65	0.59	0.69	0.49	0.87	0.56	0.62
		120	0.95	0.76	0.91	0.91	0.75	0.91	0.89	0.82	0.78	0.95
	Yeasts + molds	48	0.38	0.35	0.35	0.24	0.12	0.12	0.12	0.97	0.41	0.40
		72	0.80	0.87	0.88	0.78	0.71	0.72	0.59	0.98	0.77	0.78
		120	0.99	0.95	0.96	0.96	0.94	0.95	0.96	0.98	0.97	0.98
Slope	Yeasts	48	0.80	0.58	0.59	0.45	0.25	0.25	0.24	1.01	0.67	0.70
		72	0.80	1.06	1.09	0.85	0.87	0.90	0.67	1.00	0.79	1.00
		120	0.98	1.06	1.08	1.06	1.07	1.10	1.07	0.99	0.96	0.99
	Molds	48	0.93	0.30	0.30	0.29	0.23	0.23	0.32	0.97	0.69	0.12
		72	0.90	0.92	0.94	0.76	0.84	0.88	0.67	0.91	0.66	0.88
		120	0.98	0.97	1.00	1.02	0.95	0.99	1.00	0.86	0.85	0.96
	Yeasts + molds	48	0.80	0.54	0.54	0.47	0.25	0.25	0.26	0.98	0.68	0.60
		72	0.88	1.03	1.04	0.83	0.94	0.95	0.73	0.99	0.75	1.04
		120	0.99	1.03	1.03	1.03	1.03	1.04	1.04	0.98	0.98	0.99
Intercept	Yeasts	48	1.13	−0.10	−0.18	0.95	0.00	−0.03	0.36	−0.14	2.00	−0.51
		72	1.07	−0.49	−0.65	0.61	−0.38	−0.53	0.62	−0.07	1.08	−0.49
		120	0.11	−0.39	−0.50	−0.35	−0.46	−0.60	−0.43	0.03	0.17	−0.01
	Molds	48	0.22	0.20	0.24	0.64	0.16	0.18	0.46	0.16	1.69	−0.02
		72	0.42	−0.08	−0.13	0.98	−0.07	−0.17	0.95	0.26	1.47	−0.53
		120	0.11	0.00	−0.12	−0.13	−0.02	−0.15	−0.14	0.40	0.44	0.08
	Yeasts + molds	48	0.89	0.06	0.09	0.98	0.20	0.21	0.54	0.07	1.98	−0.42
		72	0.61	−0.41	−0.45	0.83	−0.39	−0.43	0.81	0.02	1.36	−0.81
		120	0.08	−0.23	−0.23	−0.20	−0.28	−0.29	−0.26	0.05	0.11	0.01

increase at 120 h of incubation compared with 72 h, the increase may not always be practically significant.

The mycological quality of foods is most often assessed by determining the total number of yeasts and molds. Table 5 lists the total yeast and mold populations detected in the 56 foods and food ingredients plated on the five enumeration media. Colonies were counted after incubating plates for 48, 72, and 120 h at 25°C. Data represent a composite of values from Tables 2 and 4. Neither yeasts nor molds were detected in 31 (55.4%), 41 (73.2%), 12 (21.4%), 11 (19.6%), and 40 (71.4%) of the products plated on RYM Petrifilm, YM Petrifilm, DRBC, APDA, and DG18, respectively, and incubated for 48 h (Table 3). Numbers decreased to 6 (10.7%), 8 (14.3%), 2 (3.6%), 1 (1.8%), and 11 (19.6%) products, respectively, when plates were incubated for 72 h and 2 (3.6%), 1 (1.8%), 0, 0, and 0 products, respectively, on plates incubated for 120 h.

Ease of detection of yeast and mold colonies on RYM and YM Petrifilm plates differed, depending on the type of food or food ingredient. Colonies developing from most products were easily detected, but homogenates of some products (e.g., raw walnut and black tea) caused a rapid change in background color to brownish purple. This change was attributed to enzyme activities intrinsic to these foods. Particles in some homogenates of cereal products, seasonings, and tea became blue before the Petrifilm plates were examined at 48 h, thereby occasionally delaying confirmation of yeasts and molds until colonies increased in size by 72 h. Other researchers have also reported that food particles in some types of foods interfere with detecting colonies on YM Petrifilm *(11, 32)*. Spreading mold colonies developing from a few of the test products on APDA and DG18 made it difficult to count other colonies.

Correlation coefficients, slopes, and intercepts for numbers of yeasts, molds, and yeasts plus molds recovered from the 56 foods and food ingredients on the five enumeration media are shown in Table 6. For samples in which no yeasts or molds (<10 CFU/g or ml) were detected, 1 CFU/g or ml (0 log CFU/g or ml) was used for calculations. Correlation coefficients for pairs of media other than DRBC versus APDA ranged from 0.11 to 0.46 for yeasts, 0.03 to 0.70 for molds, and 0.12 to 0.41 for yeasts plus molds recovered on plates incubated for 48 h; these values were 0.97, 0.93, and 0.97 for yeast, mold, and yeast plus mold counts, respectively, for DRBC versus APDA. Correlation coefficients approached acceptable levels on plates incubated for 72 h. Values were 0.92 to 0.99, 0.75 to 0.95, and 0.94 to 0.99 for yeasts, molds, and yeast plus molds, respectively, when plates were incubated for 120 h.

TABLE 7. *Coefficients of variation in the number of yeasts, molds, and yeasts plus molds recovered from 56 foods and food ingredients on mycological enumeration media incubated for 48, 72, and 120 h at 25°C*

Microorganisms	Incubation time (h)	Coefficient of variation (%)				
		RYM	YM	DRBC	APDA	DG18
Yeasts	48	9.7	2.1	30.4	28.0	23.0
	72	24.4	14.9	38.3	35.2	25.7
	120	23.2	19.0	42.9	36.2	30.1
Molds	48	17.7	10.5	40.1	38.8	4.5
	72	34.4	39.0	38.3	34.3	39.5
	120	25.6	33.5	34.7	32.3	32.8
Yeasts + molds	48	17.7	8.1	30.0	27.1	12.5
	72	28.0	30.8	28.9	26.4	28.7
	120	24.3	27.2	26.4	24.2	23.8

Values for slopes and intercepts reflect corresponding low or high r^2 values.

Coefficients of variation in the yeast, mold, and yeast plus mold counts detected in the 56 foods and food ingredients on the five test media are shown in Table 7. Values were lower on media incubated for 48 h compared with those for plates incubated for 72 or 120 h; however, regardless of the incubation time or recovery medium, coefficients of variation were high: 19.0 to 42.9% for yeast counts, 25.6 to 34.7% for molds, and 23.8 to 27.2% for yeasts plus molds on media incubated for 120 h. These high values can be attributed to large differences in populations of yeasts and molds in test foods, the broad diversity of yeasts and molds likely to be present, and differences in requirements for resuscitation of stressed cells and colony development.

Figure 1 shows the relationship between RYM Petrifilm, YM Petrifilm, DRBC, APDA, and DG18 for supporting colony development by yeasts plus molds in the 56 test foods and food ingredients. These plots further illustrate that the performance of RYM Petrifilm versus the other media improves as the incubation time increases between 48 to 72 h and, again, between 72 and 120 h. These observations reflect, in part, the need for longer incubation times for colony development by yeasts and molds in low-a_w foods.

Counts obtained at each incubation time were compared to determine the time at which decisions might be made concerning actionable levels in a commercial setting. Comparisons of significant differences in counts at 72 versus 48 h, 120 versus 72 h, and 120 versus 48 h for each enumeration medium are shown in Table 8. For yeasts plus molds, 72-h counts were 23.3 to 50.0% higher than 48-h counts depending on the medium, and 120-h counts were 14.3 to 48.2% higher than 72-h counts and 37.5 to 78.6% higher than 48-h counts. Regardless of the target microorganism, DRBC and APDA were superior in supporting early colony development, thereby resulting in lower numbers of samples with large differences in counts at each incremental increase in incubation time. The incubation time at which an

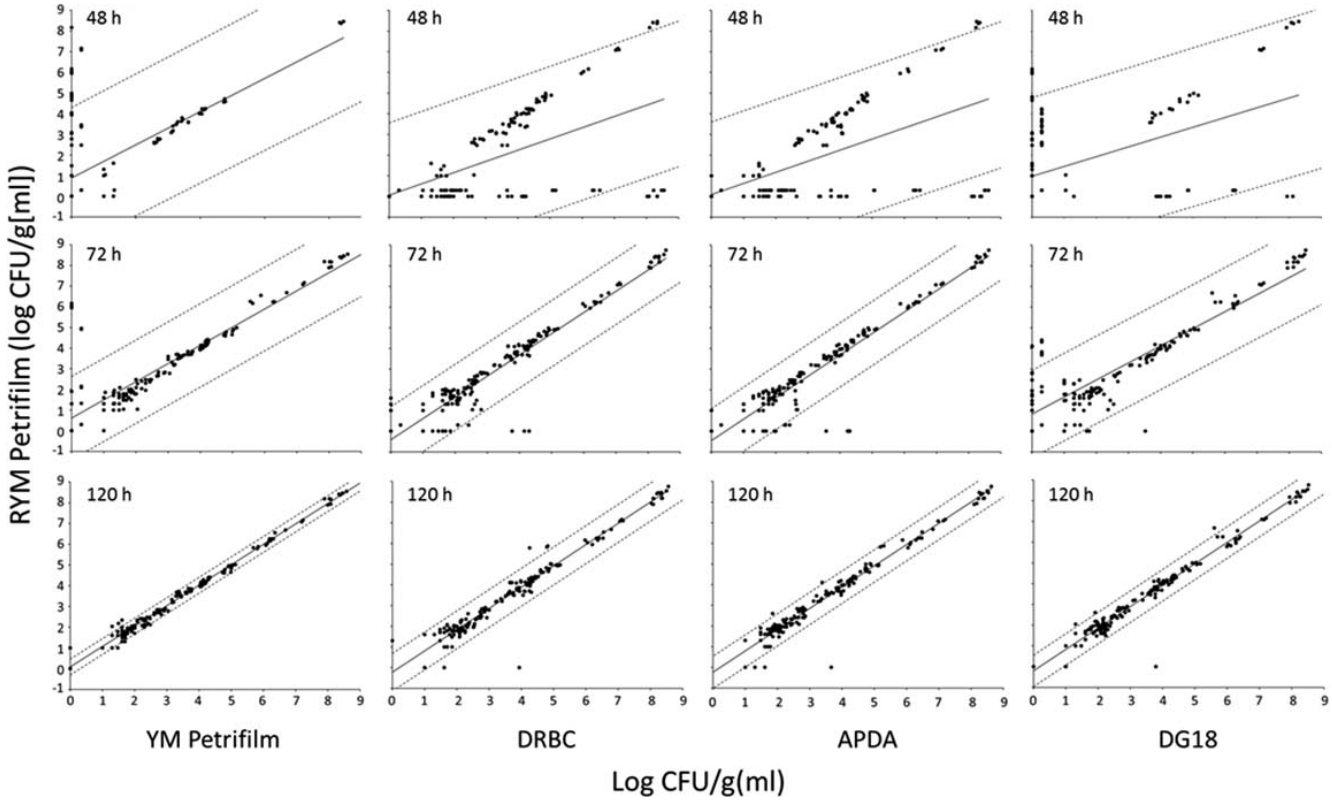

FIGURE 1. *Relation between yeast plus mold populations (log CFU/g or ml) recovered on RYM Petrifilm incubated for 48, 72, and 120 h versus populations recovered on YM Petrifilm, DRBC, APDA, and DG18. Data represent composites of values from 56 foods and food ingredients and are indicated by linear regression lines (solid lines) with 95% confidence limits (dashed lines). For samples in which no yeasts or molds were detected (<10 CFU/g or ml), 1 CFU/g or ml was used for calculations.*

TABLE 8. *Comparison of mycological media for enumerating yeasts, molds, and yeast plus molds from 56 foods and food ingredients after incubating for 48, 72, and 120 h at 25°C*

No. of foods or ingredients[a]	Microorganisms	Comparison of incubation time (h)	No. (%) of samples[b]				
			RYM	YM	DRBC	APDA	DG18
34	Yeasts	72 vs 48	15 (44.1)	17 (50.0)	4 (11.2)	4 (11.8)	10 (29.4)
		120 vs 72	3 (8.8)	9 (26.5)	3 (8.8)	3 (8.8)	8 (23.5)
		120 vs 48	18 (52.9)	22 (64.7)	7 (20.6)	6 (17.6)	17 (50.0)
43	Molds	72 vs 48	19 (44.2)	18 (41.9)	12 (27.9)	17 (39.5)	16 (37.2)
		120 vs 72	14 (32.6)	19 (44.2)	10 (23.3)	9 (20.9)	23 (53.5)
		120 vs 48	29 (67.4)	31 (72.1)	18 (41.9)	23 (53.5)	36 (83.7)
56	Yeasts + molds	72 vs 48	25 (44.6)	28 (50.0)	13 (23.2)	17 (30.4)	21 (37.5)
		120 vs 72	13 (23.2)	27 (48.2)	9 (16.1)	8 (14.3)	25 (44.6)
		120 vs 48	35 (62.5)	44 (78.6)	21 (37.5)	23 (41.1)	42 (75.0)

[a] Yeasts, molds, and yeasts plus molds were detected in or on 34, 43, and 56, respectively, foods and food ingredients.
[b] Values indicate the number of samples out of 34 (yeasts), 43 (molds), or 56 (yeasts plus molds) food and food ingredients in which counts were significantly higher ($P \leq 0.05$) after 72 h compared with 48 h, after 120 h compared with 72 h, and after 120 h compared with 48 h. Numbers in parentheses indicate percentage of samples with significantly higher counts after the longer incubation time.

actionable level for yeasts and molds in a particular food can be determined is up to the manufacturer of that food, but decisions should always be based on the type of food in combination with the enumeration medium.

To give a better sense of the performance of RYM Petrifilm versus the other media at each incubation time, the number of times populations of yeasts, molds, and yeasts plus molds detected on RYM Petrifilm were significantly ($P \leq 0.05$) higher or lower than those on YM Petrifilm, DRBC, APDA, and DG18 was calculated (Table 9). The performance of RYM Petrifilm was generally better than that of YM Petrifilm and DG18 but was inferior to the performance of DRBC and APDA regardless of incubation time.

A quantitative comparison of media for enumerating yeasts plus molds is shown in Table 10. The number and percentage of samples in which counts obtained on each pair of media were equivalent or significantly different are listed. RYM Petrifilm performed marginally better than did YM Petrifilm and DG18 but not as well as DRBC and APDA when colonies were counted after incubating media for 48 or 72 h. Recovery on YM Petrifilm was equivalent or better than that on DG18. Significant differences in performance of media were less evident as the incubation time was extended from 48 to 72 h and from 72 to 120 h. The general order of performance of media for supporting colony development by yeasts and molds naturally present in 56 foods and food ingredients differed depending on the plate incubation time: DRBC = APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 at 48 h, DRBC > APDA > RYM Petrifilm > YM Petrifilm ≥ DG18 at 72 h, and DRBC > APDA > RYM Petrifilm = YM Petrifilm > DG18 at 120 h. This order is altered when considering specific types of foods. For example, RYM Petrifilm compared favorably with DRBC and APDA in detecting yeasts and molds in some of the high-a_w foods within 48 h.

Differences in performance of the mycological media evaluated in this study for supporting colony development by the diverse mycobiota likely to be naturally present on or in the 56 test products were expected. The prevalence of field and storage molds in cereal products, in contrast for example to spoilage yeasts typically found in fruits and fruit products, brings with it differences in nutrient, pH, and a_w requirements for fungal growth. DG18 agar (a_w 0.95) was developed to enumerate nonfastidious xerophilic molds in low-a_w foods containing rapidly growing *Eurotium* species *(20),* but this medium also supports slow growth of other

TABLE 9. *Comparison of RYM Petrifilm with other mycological enumeration media for recovering yeasts, molds, and yeasts plus molds from 34, 43, and 56, respectively, foods and food ingredients after incubating plates for 48, 72, and 120 h at 25°C*

No. of foods or ingredients	Microorganisms	Incubation time (h)	No. of samples with significantly ($P \leq 0.05$) higher/lower counts on RYM than on:			
			YM	DRBC	APDA	DG18
34	Yeasts	48	9/0	1/11	1/15	7/5
		72	6/0	3/9	1/8	5/5
		120	0/1	3/6	2/4	3/4
43	Molds	48	1/0	0/19	0/9	7/0
		72	3/0	0/19	0/12	9/1
		120	2/0	1/13	2/9	1/6
56	Yeasts + molds	48	10/0	1/27	1/24	12/5
		72	7/0	3/23	1/17	10/6
		120	1/1	3/18	1/11	2/8

TABLE 10. *Quantitative comparison of media for enumerating yeasts plus molds*

Medium A	Medium B	Incubation time (h)	No. (%) of samples without or with significant population differences in paired media[a]		
			A = B	A > B	A < B
RYM Petrifilm	YM	48	46 (82.1)	10 (17.9)	0 (0)
		72	49 (87.5)	7 (12.5)	0 (0)
		120	54 (96.4)	1 (1.8)	1 (1.8)
RYM Petrifilm	DRBC	48	28 (50.0)	1 (1.8)	27 (48.2)
		72	30 (53.6)	3 (5.4)	23 (41.1)
		120	35 (62.5)	3 (5.4)	18 (32.1)
RYM Petrifilm	APDA	48	31 (55.4)	1 (1.8)	24 (42.9)
		72	38 (67.9)	1 (1.8)	17 (30.4)
		120	44 (78.6)	1 (1.8)	11 (19.6)
RYM Petrifilm	DG18	48	39 (69.6)	12 (21.4)	5 (8.9)
		72	40 (71.4)	10 (17.9)	6 (10.7)
		120	46 (82.1)	2 (3.4)	8 (14.3)
YM Petrifilm	DRBC	48	21 (37.5)	0 (0)	35 (62.5)
		72	27 (48.2)	1 (1.8)	28 (50.0)
		120	36 (64.3)	1 (1.8)	19 (33.9)
YM Petrifilm	APDA	48	23 (41.1)	0 (0)	33 (58.9)
		72	31 (55.4)	1 (1.8)	24 (42.8)
		120	42 (75.0)	1 (1.8)	13 (23.2)
YM Petrifilm	DG18	48	39 (69.6)	8 (14.3)	9 (16.1)
		72	35 (62.5)	10 (17.8)	11 (19.6)
		120	39 (69.6)	5 (8.9)	12 (21.4)
DRBC	APDA	48	41 (73.2)	9 (16.1)	6 (10.7)
		72	42 (75.0)	8 (14.3)	6 (10.7)
		120	47 (83.9)	7 (12.5)	2 (3.6)
DRBC	DG18	48	27 (48.2)	29 (51.8)	0 (0)
		72	32 (57.1)	22 (39.3)	2 (3.6)
		120	41 (73.2)	11 (19.6)	4 (7.1)
APDA	DG18	48	28 (50.0)	28 (50.0)	0 (0)
		72	34 (60.7)	21 (37.5)	1 (1.8)
		120	46 (82.1)	6 (10.7)	4 (7.1)

[a] Values are the number (percentage) of the 56 foods and food ingredients analyzed in which populations of yeasts plus molds were not significantly different ($P > 0.05$) as determined using the two media being compared (A = B) or were significantly different ($P \leq 0.05$) as determined using the two media being compared (A > B or A < B).

yeasts and molds. The size of spreading mold colonies is restricted on DRBC *(21),* but this medium, along with APDA, RYM Petrifilm, and YM Petrifilm, is not formulated to recover yeasts and molds that are strict xerophiles. Recovery of sublethally heat-stressed yeasts *(23, 26)* and yeasts from chilled meat and dairy products *(3)* and fruit purees *(9)* is affected by the pH of the enumeration medium, and recovery of desiccated fungal cells is known to be affected by composition of the recovery medium *(4–7).* Thus, considering the diversity in types of foods examined in our study, it is not surprising that differences in the detection of some of the fungal contaminants, in the ability to resuscitate, and in the rates of colony development were noted among the test media.

Our observations on the performance of RYM Petrifilm are not in agreement with those reported by others *(2, 12, 13, 28),* who found similar yeast and mold counts on RYM Petrifilm and reference media at incubation times of 48, 60, or 72 h. This discrepancy is attributed largely to use in our study of only naturally contaminated foods, many of which very likely contained indigenous stressed fungal cells, rather than including foods artificially inoculated with healthy fungal cells. Fungal cells exposed to stress environments for several weeks or even months require a longer incubation time to form visible colonies regardless of the enumeration medium. These cells are able to resuscitate on some media more rapidly than on others.

Aside from differences in the performance of the enumeration media at each incubation time, RYM and YM Petrifilms offer an advantage over traditional media in that they do not require preparation and, unlike traditional agar plates that may be examined at 48 or 72 h before final counts are made at 120 h, the possible development of satellite colonies is not an issue. However, picking colonies from Petrifilm plates for identification is more difficult than picking colonies from traditional media on which food homogenates are spread plated. No single medium can be used to enumerate all yeasts and molds in all foods. The incubation time at which actionable levels of yeasts and molds naturally present in a food or food ingredient can be determined, in terms of acceptable or unacceptable quality, will depend greatly on the composition, pH, and a_w of the

product being examined, the formulation of the enumeration medium, and the limits set by the manufacturer and regulatory agencies.

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