# Alternative to Laboratory Cyclone Equipment for Pulping Methods

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**Abstract**
As part of its larger mandate, the FDA examines certain products for the presence of excessive mold. Currently, only one FDA field laboratory possesses the AOAC specified pulper (Cyclone). This severely limits the analytical capacity of the FDA for products analyzed by Howard Mold Count (HMC). The goal of this study was to determine if a commercially available pulper could prepare product for HMC analysis. Two commercially available pulpers, Kuvings and Robot Coupe, were evaluated across multiple treatment levels on canned tomatoes and frozen raspberries to test for a suitable replacement for the Cyclone. These matrices were chosen because they represent the highest percentage (>70%) of products analyzed by HMC. Per AOAC Official Method 984.29 Howard Mold Counting, analysts scored fields either positive or negative for mold. Data were analyzed using a two-sample Mann-Whitney U test with an alpha = 0.05 (two-tailed). The Kuvings yielded fewer positive fields than the Cyclone at two of three treatment levels (Tomato-High: p = 0.04, Raspberry-Blank: p = 0.00001). Results between the Cyclone and Robot Coupe were similar across all three treatment levels (p = 0.06 – 0.97). These findings suggest the Robot Coupe is a suitable substitute for the Cyclone pulper for use with the HMC method.

**Key Words**
Pulper, Mold, Howard Mold Count (HMC), Raspberry, Tomato, AOAC, Cyclone, Kuvings, Robot Coupe

## Introduction

As part of its larger mandate, the FDA examines certain products for the presence of excessive mold. The development of the laboratory Cyclone, a custom designed pulper (per AOAC 945.75B(g); AOAC, 2023), started in 1932 and the finished version was sent to the various FDA laboratories in 1944 (Wildman, 1947). Currently, only one FDA field laboratory possesses a working Cyclone. This severely limits the analytical capacity of the FDA for many products that are analyzed by Howard Mold Count (HMC), such as canned tomatoes and frozen drupelet berries. Further, no other internal or external laboratories can meet AOAC specifications. To address this situation, two commercially available, off-the-shelf pulpers were evaluated against the current standard laboratory Cyclone.

Canned tomatoes and frozen raspberries were chosen for this evaluation because they are currently the most common (>70%) products analyzed by HMC at the FDA. They also represent biologically distinct structures, which may differentially affect pulping. Tomatoes are smooth-skinned fruits containing numerous seeds held in a jelly-like material. Tomato seeds are covered with polygonal cells and have numerous false hairs. Raspberries are an aggregated fruit containing numerous drupelets. The fruit has hard seeds, and the exposed surface of the berry has numerous hairs. They also have relatively long styles. The hairs are thin walled and have a kinky appearance (Winton & Winton, 1935). These matrices evaluated the range of biological structures the equipment must process for HMC analysis.

## Materials and Methods

### Equipment

Cyclone, with 0.5 mm screen, Cefaly/Century Electric (as specified in AOAC 945.75(B)(g); AOAC, 2023) See Appendix 1 for photo.

Kuvings EVO820 Whole slow juicer, with juicing strainer screen (0.5334 mm top & 0.4572 mm bottom), Kuvings USA, 236 Egidi Dr, Suite C, Wheeling, IL 60090 – www.kuvingsusa.com See Appendix 2 for photo.

Robot Coupe C80, with 0.5 mm screen, Robot Coupe U.S.A., Inc. P.O. Box 16625 South Perkins St., Ridgeland, MS 39157 – www.robotcoupeusa.com See Appendix 3 for photo.

No. 8 (2.36 mm/0.0937) sieve, 12-inch diameter, full- height, Gilson Company, Inc. 7975 North Central Dr, Lewis Center, OH 43035 – www.globalgilson.com

S60 Howard Cell Counting Cell (02C00420), Graticules Optics Limited, 17-19 Morley Road, Tonbridge, Kent, TN9 1RN, UK – www.graticulesoptics.com See Appendix 4 for photo.

K20 Segewick Rafter Counting Chamber (02C00419), Graticules Optics Limited, 17-19 Morley Road, Tonbridge, Kent, TN9 1RN, UK – www.graticulesoptics.com See Appendix 4 for photo.

Thermo Scientific 295 Samco Special Purpose non-sterile Padl-Pet 50 µl drop size transfer pipette. (13-711-46), Thermo Fisher Scientific – www.fishersci.com See Appendix 4 for photo.

Falcon 50 mL sterile polypropylene conical tube 30 x 115 mm style (352070), Corning Science – www.corning.com/lifesciences

Falcon 100 mL serological pipet (357600), Corning Science – www.corning.com/lifesciences

Falcon 100 x 15 mm sterile polystyrene Petri dish, (51029), Corning Science – www.corning.com/lifesciences.

Hampton Bay desk lamp, Model 1127 Series, Portable Luminaire, 120V~60Hz 420mA 27W (057), or equivalent, to visualize Newton's Rings, Hampton Bay – www.thehamptonbay.com

Leica DM 2000 compound microscope fitted with Howard mold counting reticles (KR-832), Klarmann Rulings, Inc., 1 Perimeter Road Unit 900, Manchester, NH 03103 – www.reticles.com

Mettler Toledo PM 15 balance, Mettler Toledo Mettler-Toledo, LLC 1900 Polaris Parkway Columbus, OH 43240 – www.mt.com/us

Zip lock poly bags, 1 gallon size

Waring Commercial Blender 700G, Model WF2212112, Waring Commercial Company, 1 Cummings Point Rd. Stamford, CT 06902 – www.waring.com/blend

Variable Autotransformer, Type 3PN1010V, Staco Energy Products Co., 2425 Technical Dr. Miamisburg, OH 45342 – www.stacoenergy.com

**Test Product**
Whole peeled tomatoes in puree, in #10 cans

Whole IQF red raspberries in 5-pound bags

**Reagents**

Formaldehyde (HCHO) 37% (BP531-500), Fisher Bioreagents – www.fishersci.com

2-Octanol 98% (A16985), Alfa Aesar – www.fishersci.com

Stabilizer solution – per AOAC 945.75C(v) (AOAC, 2023)

Spike mold – naturally occurring mold from the whole canned tomato product (whole tomatoes and packing medium) was grown on Petri plates. The product was blended and a thin layer (~5 mm depth/ ~20 mL volume) was poured into 100 mm x 15 mm Petri plates and left for approximately one week under ambient conditions inside a fume hood (Figure 1). Once a uniform mat of mold mycelia covered the entire surface (Figure 1C), the plates were placed in plastic bags and stored in the freezer (~-20° C) until used for spike material.

**Procedures**

Tomatoes:

All tomato product was composited at the start. The composite consisted of randomly selected cans mixed to create the starting material for each treatment level prior to adding the spike mold material. To create the composite, all selected cans were opened, packing medium drained, then tomatoes from all cans commingled. Once mixed, subsamples were weighed and separated into individual zip lock bags prior to spiking and subsequent pulping. By creating a composite, each treatment level had an equivalent baseline of mold filaments.

To evaluate pulper performance, one blank and two treatment levels were evaluated. The blank contained only tomato composite without spiked mold. Four replicates of low spike inoculum, and four replicates of high spike inoculum per pulper were prepared by spiking mold into tomato composite.

Each individually spiked sub was processed through one of the three pulpers: Cyclone, Kuvings, or Robot Coupe. To remove potential analytical bias, pulped subsamples were randomized and blinded with respect to treatment level and pulper. Each subsample was prepared per AOAC 945.90 (AOAC, 2023) and AOAC 984.29 (AOAC, 2023). Once all subsamples were evaluated, the identity key was applied prior to data analysis.

1. Process for spiking subsamples:
    a. Preparation of spike material
        i. Contents from ~20 frozen Petri plates were added to a blender jar and thawed at room temperature
        ii. Once thawed, contents were blended for 15 seconds until a homogenous mixture was obtained
        iii. This process was repeated to obtain an adequate amount of spike material for all treatments (240 plates minimum). All blended spike

material was added to one container and mixed before adding to subsamples.

b. Formation of composite
    i. Randomly selected 27 cans of tomatoes from lot
    ii. Drained tomatoes from each can for 2 minutes using #8 (0.0937"/2.36 mm) sieve
    iii. Gently rinsed any remaining packing medium off tomatoes with tap water
    iv. Drained tomatoes for an additional 2 minutes using #8 sieve
    v. Combined the rinsed and drained tomatoes and mixed thoroughly, but gently, by hand to minimize breakage
    vi. Distributed whole drained, rinsed tomatoes into labeled zip lock bags per treatment as follows:
        1. Blank x 3 – 1000 g each
        2. Low x 12 – 900 g each
        3. High x 12 – 600 g each
c. Spiking subsamples
    i. Pipetted appropriate amount of spike material into each subsample as follows:
        1. Blank – no spike material
        2. Low – 100 mL spike material
        3. High – 400 mL spike material
    ii. Stored all subsamples in freezer (~-20° C) until ready to pulp

2. Pulping subsamples
    a. Removed subsamples from freezer and thawed at room temperature
    b. Once thawed, subsamples were pulped
3. Preservation of pulped material and random number assignment
    a. Well-mixed pulped material was aliquoted into labeled 50-mL centrifuge tubes
    b. Approximately 5 drops of 37% HCHO and 5 drops of 2-octanol were added to each 50-mL centrifuge tube containing pulped material
    c. Randomly generated numbers were assigned to each tube by an individual not otherwise involved with study so the analysts reading the slides would be blinded to both the treatment level and pulper
4. Howard Mold Counts per AOAC 984.29 (AOAC, 2023)
    a. Analysts: four analysts independently performed HMC
    b. Equipment utilized
        i. Leica DM 2000 compound microscope fitted with HMC reticles
        ii. Graticules S60 (circular) HMC cell
        iii. Graticules K20 cover glass
        iv. 50 µL disposable transfer pipet
    c. Method specification (not described in AOAC)
        i. Used the 50 µL disposable transfer pipet to deliver one drop of pulped material to the HMC cell and spread it evenly across the cell surface

(using the opposite paddle-shaped end of the transfer pipet) before placing the cover glass on top

ii. Used a new transfer pipet for each slide preparation
iii. Recorded the results on a hardcopy data sheet with initials and date for each slide count

Raspberries:
A single composite of raspberries was created from randomly selected 5-lb. bags to create the starting material for all the subsamples. Once mixed, subsamples were weighed and separated into individual zip lock bags prior to pulping.

Preliminary counts on the raspberries showed high levels of mold present (>60% positive fields). To evaluate pulper performance for raspberries, four replicates of a single treatment level were evaluated. Because no spike material was added to the raspberry composite, this treatment level was labeled as “Blank”.

Each individual subsample was processed through one of the 3 pulpers: Cyclone, Kuvings, or Robot Coupe. To remove analytical bias, pulped subsamples were randomized and blinded with respect to pulper. Each subsample was prepared per AOAC 955.47 (AOAC, 2023) and AOAC 984.29 (AOAC, 2023). Once all subsamples were evaluated, the identity key was applied prior to data analysis.

1. Formation of composite
    a. Randomly selected eight 5-lb bags of frozen raspberries from lot
    b. While still frozen, contents of all eight bags were mixed thoroughly, but gently, by hand (to minimize breakage) to form a composite
    c. Distributed 1000 g of frozen raspberries into zip lock bags to create 12 individual subsamples
    d. Stored subsamples in freezer (~-20° C) until ready to pulp
2. Pulping subsamples
    a. Removed subsamples from freezer and thawed at room temperature for approximately 1.5 hours
    b. Once thawed, subsamples were pulped
3. Preservation of pulped material and random number assignment
    a. After pulped material was thoroughly mixed, 50 ml stabilizer solution was added to 25g of pulped material and mixed well
    b. Mixture was then aliquoted into labeled 50 mL centrifuge tubes
    c. 5 drops of 2-octanol were then added to each 50 mL tube
    d. Randomly generated numbers were assigned to each tube by an individual not otherwise involved with study so the analysts reading the slides would be blinded to both the treatment level and pulper
    e. Tubes were stored in refrigerator (~4° C) until ready to analyze
4. Performing Howard Mold Counts as per AOAC 984.29 (AOAC, 2023)

a. Analysts: four analysts independently performed HMC
b. Equipment utilized
    i. Leica DM 2000 compound microscope fitted with HMC reticles
    ii. Graticules S60 (circular) HMC cell
    iii. Graticules K20 cover glass
    iv. 50 µL disposable transfer pipet
c. Method specification (not described in AOAC)
    i. Used the 50 µL disposable transfer pipet to deliver one drop of pulped material to the HMC cell and spread it evenly across the cell surface (using the opposite paddle-shaped end of the transfer pipet) before placing the cover glass on top
    ii. Used a new transfer pipet for each slide preparation
    iii. Recorded the results on a hardcopy data sheet with initials and date for each slide count

**Data**

Per AOAC 984.29 (AOAC, 2023), each analyst prepared two separate slide mounts per subsample and scored 25 fields from each slide (50 total fields). Each field was scored either positive or negative, resulting in a range from 0 - 50 positive fields. Summary statistics are available in Table 1.

**Statistical Evaluation**

The Robot Coupe and Kuvings pulpers were individually evaluated against the Cyclone across the three different treatment levels. We hypothesized that the Robot Coupe and Kuvings recoveries would not be statistically different from the Cyclone within each treatment level. To evaluate this hypothesis, we used a two sample Mann-Whitney U test (Mann & Whitney, 1947) with an alpha = 0.05 (two-tailed).

**Results and Discussion**

Mold counts were statistically similar between the Cyclone and Robot Coupe across the three treatment levels (Table 2; Figure 2). Mold counts were similar between the Cyclone and Kuvings at the Tomato-Low treatment level ($p = 0.24$; Table 2; Figure 2); however, the Kuvings mold counts were lower than the Cyclone at the Tomato-High and Raspberry-Blank treatment levels ($p = 0.04$ and $p = 0.00001$, respectively; Table 2; Figure 2). The Kuvings is the slowest spinning pulper (50 rpm vs. 1725 - 1750 rpm for Cyclone and Robot Coupe) and needed to be unclogged multiple times while pulping the Tomato-High and Raspberry-Blank treatment levels. This, coupled with the slightly smaller bottom screen hole diameter (0.4572 mm vs 0.5 mm) might explain the lower counts observed at these treatment levels when compared to the Cyclone. Taken together, the results suggest that the Kuvings is unsuitable as a substitute for the Cyclone pulper when preparing samples for HMC analysis.

A mean/median difference (Table 1) of approximately four positive fields was observed between the Cyclone and Robot Coupe in the Raspberry-Blank treatment level resulting in a marginal statistical difference ($p = 0.06$). This amount of variation is acceptable when considering that two slide counts from the same subsample can vary by up to three positive fields before additional counts must be completed (ORA Lab Manual (IV)4, 2022). Further, the counts from the Cyclone Raspberry-Blank treatment would have resulted in two Laboratory Classification (LC; ORA Lab Manual (III)3, 2023)) 2s and two LC3s, while the counts from the Robot Coupe Raspberry-Blank treatment would have resulted in three LC2s and one LC3, with all counts centering around the defect action levels (FDA DALs, 2024) at 60% positive fields (50 – 82% for Robot Coupe, 50 – 86% for Cyclone). The counts from the Kuvings Raspberry-Blank treatment would have resulted in four LC1s with all counts ≤60%, suggesting lack of suitability as a substitute for the Cyclone pulper when used for raspberries.

Consistent results were obtained using a Poisson generalized linear modeling approach and a two-way ANOVA (outputs not included). Per consultation with a statistician, these approaches were explored to account for potential independence issues encountered with multiple samples being drawn from a single tube. The Poisson generalized linear modeling approach was used to evaluate the individual and interaction effects of Analyst (Analyst 1, 2, 3, and 4) and Equipment (Cyclone, Kuvings, and Robot Coupe) on the % of positive fields (0 - 100). The Tomato-Blank data were excluded due to a small sample size, and treatment was not included in the model as we were not interested in comparing between treatments (i.e., comparing Tomato-Low to Raspberry-Blank). The results for this model supported that the Kuvings counts were lower than the Cyclone ($p = 0.01$) while the Robot Coupe and Cyclone were consistent ($p = 0.872$). Additionally, one analyst (Analyst 4; Figure 3) had lower counts ($p = 0.02$) overall than the rest of the analysts, but these lower counts were not present when incorporating the interaction effect of analyst and equipment ($p = 0.33 – 0.98$). Despite having lower counts, Analyst 4 had consistent results between pulpers. Variation was expected between analysts (and even between counts from an individual analyst), and when looking at the interaction plot, we can see that one analyst consistently found more mold (Analyst 3; Figure 3) while one consistently found less (Analyst 4; Figure 3). Additionally, the ANOVA model demonstrated that no statistical difference ($p = 0.85$) existed between the mold counts from the Cyclone and the Robot Coupe.

Based on the consistent mold counts at all three treatment levels, the Robot Coupe C80 with the 0.5 mm screen is a suitable replacement for the Cyclone. Additionally, the lower counts at the Tomato-High and Raspberry-Blank treatment levels suggest that the Kuvings EVO820 with the juicing strainer screen is not a suitable replacement for the Cyclone.

**Acknowledgment**
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**Tables**

Table 1. Summary statistics based on the percent positive fields (out of 50 total fields) by treatment and equipment.

| | | Percent positive fields | | |
|---|---|---|---|---|
| **Treatment** | **Equipment** | **Mean** | **SD** | **Median** |
| Tomato - blank | Robot Coupe | 4.6 (n = 4) | 3.0 | 4.0 |
| Tomato - blank | Cyclone | 5.0 (n = 4) | 1.2 | 5.0 |
| Tomato - blank | Kuvings | 10.0 (n = 4) | 10.5 | 7.0 |
| Tomato - low | Robot Coupe | 77.2 (n = 16) | 14.3 | 73.0 |
| Tomato - low | Cyclone | 75.8 (n = 16) | 12.9 | 74.0 |
| Tomato - low | Kuvings | 69.8 (n = 16) | 16.7 | 70.0 |
| Tomato - high | Robot Coupe | 89.2 (n = 16) | 10.9 | 92.0 |
| Tomato - high | Cyclone | 88.8 (n = 16) | 11.0 | 89.0 |
| Tomato - high | Kuvings | 78.8 (n = 16) | 14.8 | 81.0 |
| Raspberry - blank | Robot Coupe | 64.8 (n = 16) | 8.9 | 64.0 |
| Raspberry - blank | Cyclone | 71.8 (n = 16) | 11.9 | 72.0 |
| Raspberry - blank | Kuvings | 43.2 (n = 16) | 11.1 | 46.0 |

Table 2. Mann-Whitney U test (Mann & Whitney, 1947) statistics and corresponding p values for the equipment comparisons across treatment levels. Asterisks (*) denote equipment that were statistically different ($p < 0.05$) from the Cyclone within the respective treatment level.

| **Treatment** | **Equipment Comparison** | **Mann-Whitney U test statistic** | **p value** |
|---|---|---|---|
| Tomato - Low | Cyclone – Robot Coupe | 129.5 | 0.97 |
| Tomato - High | Cyclone – Robot Coupe | 121.5 | 0.82 |
| Raspberry - Blank | Cyclone – Robot Coupe | 178.5 | 0.06 |
| Tomato - Low | Cyclone – Kuvings | 159.5 | 0.24 |
| Tomato - High | Cyclone – Kuvings | 183 | 0.04* |
| Raspberry - Blank | Cyclone – Kuvings | 243.5 | 0.00001* |

**Figures**

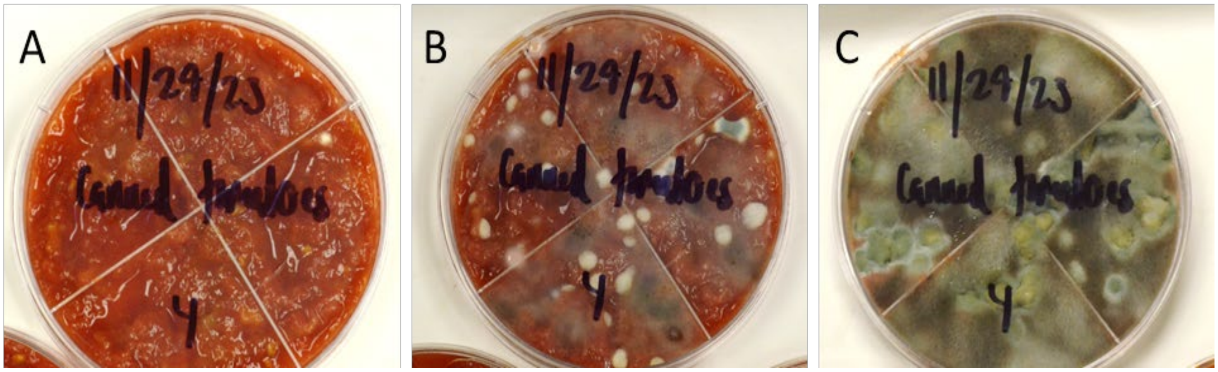



Figure 1. Progression of mold growth on one plate over time in blended whole canned tomato product one day after pouring (A), two days after pouring (B), and five days after pouring (C).

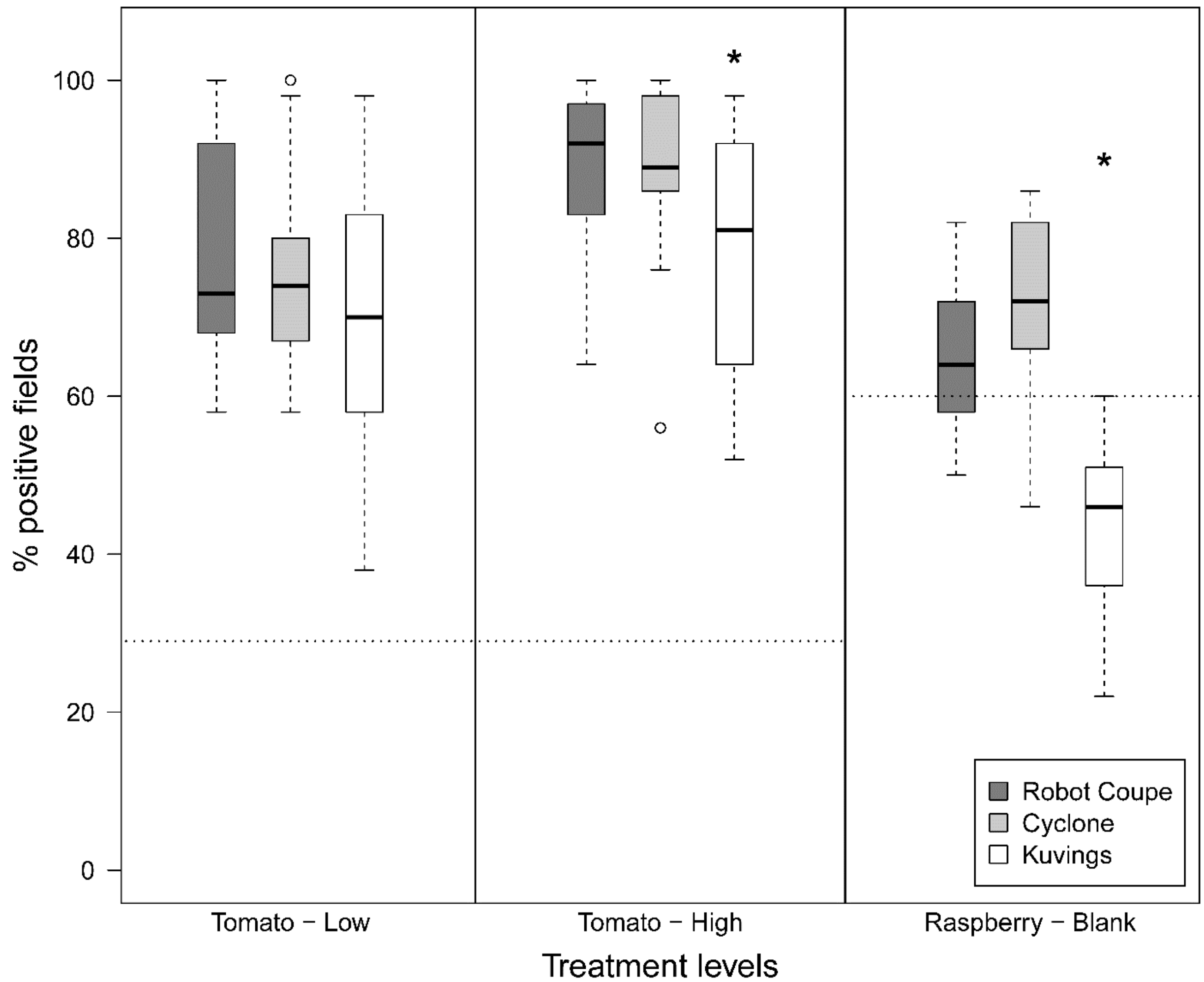



Figure 2. Boxplot comparing equipment (Robot Coupe, Cyclone, Kuvings) within the three treatment levels based on the percent of positive fields (out of 50 total fields). Asterisks (*) denote equipment that were statistically different ($p < 0.05$) from the Cyclone within the respective treatment level. Circles (o) denote outliers in the data. The horizontal dotted lines denote the Defect Action Levels (FDA DALs, 2024) for each product: 29% for canned tomato products and 60% for frozen raspberries.

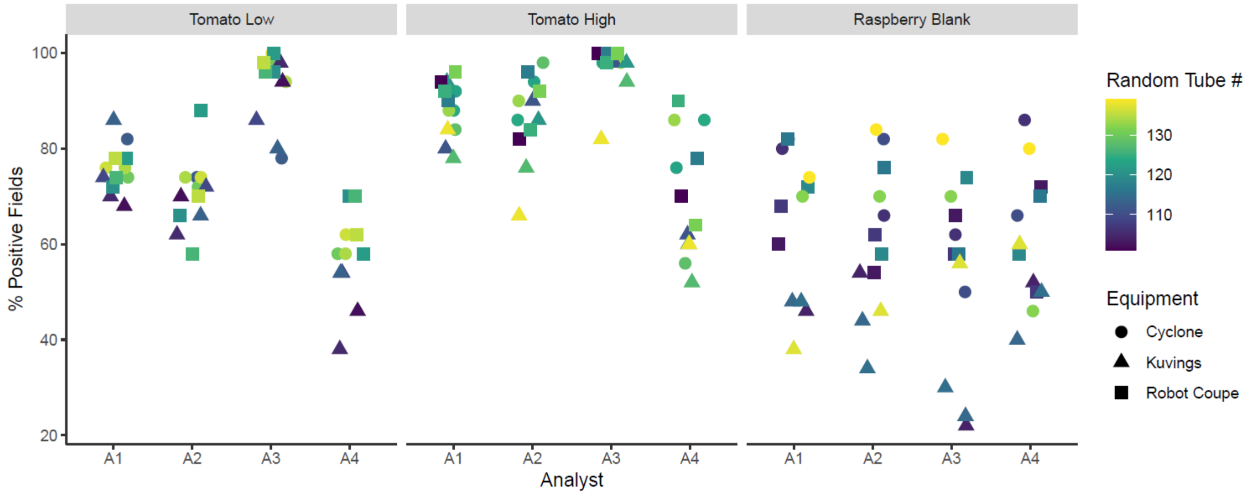
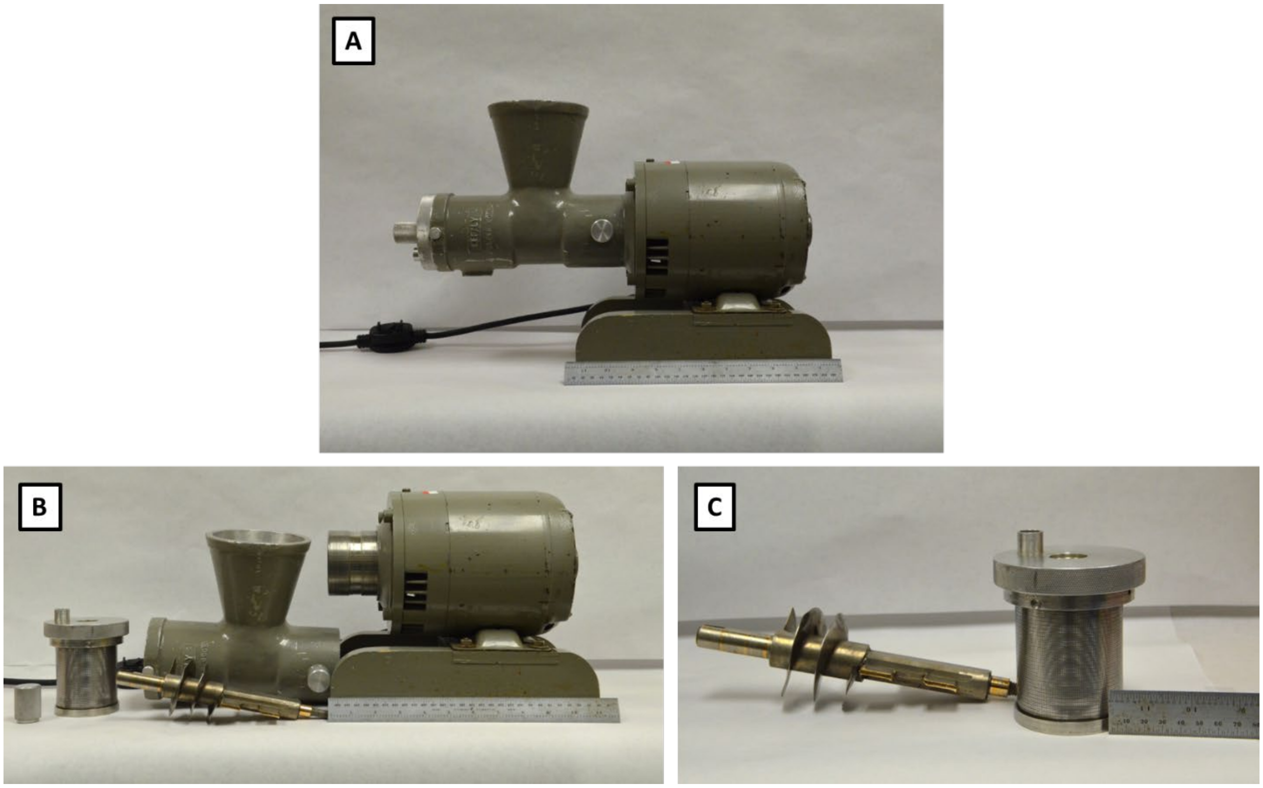


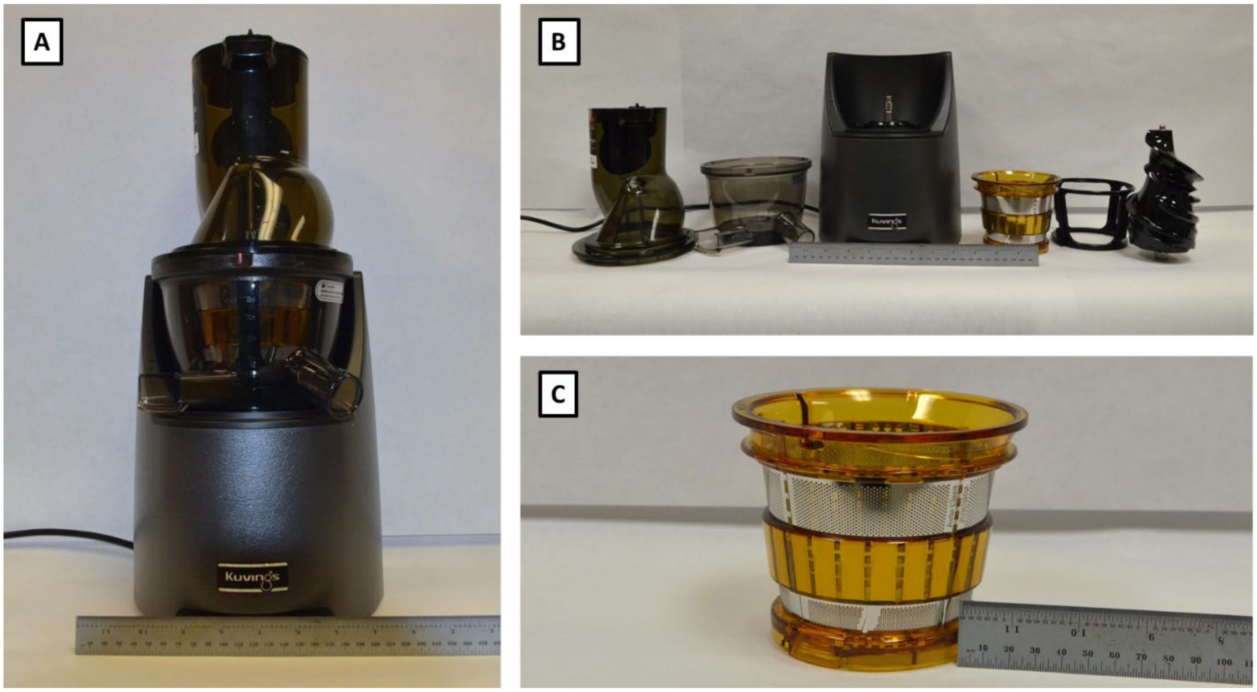

Figure 3. Interaction plot showing the variation in percent positive fields (y-axis) based on random tube number (color), Analyst (x-axis), and Equipment (shape). There were 36 total tubes, 12 per treatment that were randomly numbered 101 – 139. These tubes are represented by the color gradient.

**Appendix 1**



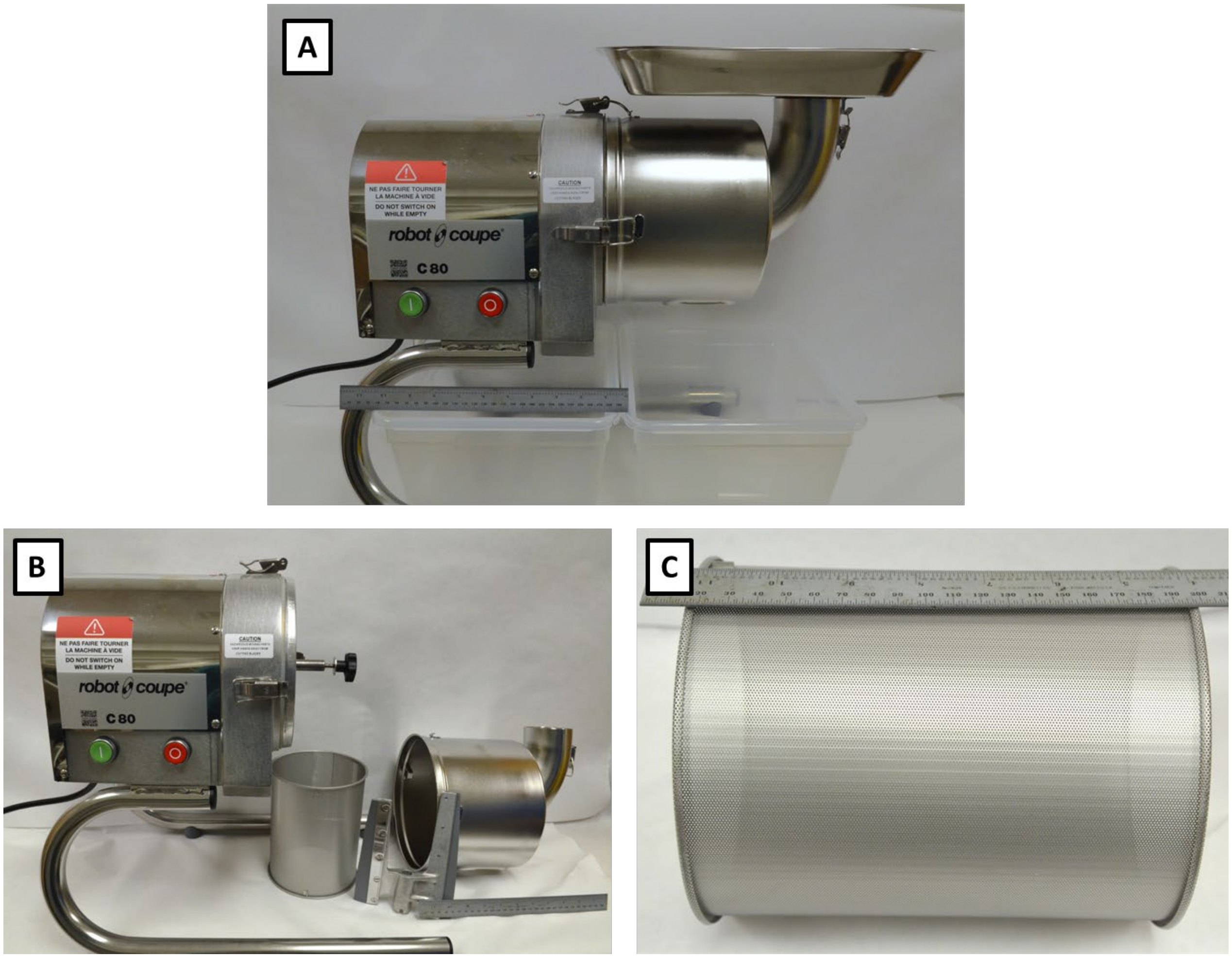

Cyclone, with 0.5 mm screen, Cefaly/Century Electric per AOAC 945.75(B)(g) (AOAC, 2023). Depicted assembled (A), disassembled (B), and just the auger and screen (C).

**Appendix 2**



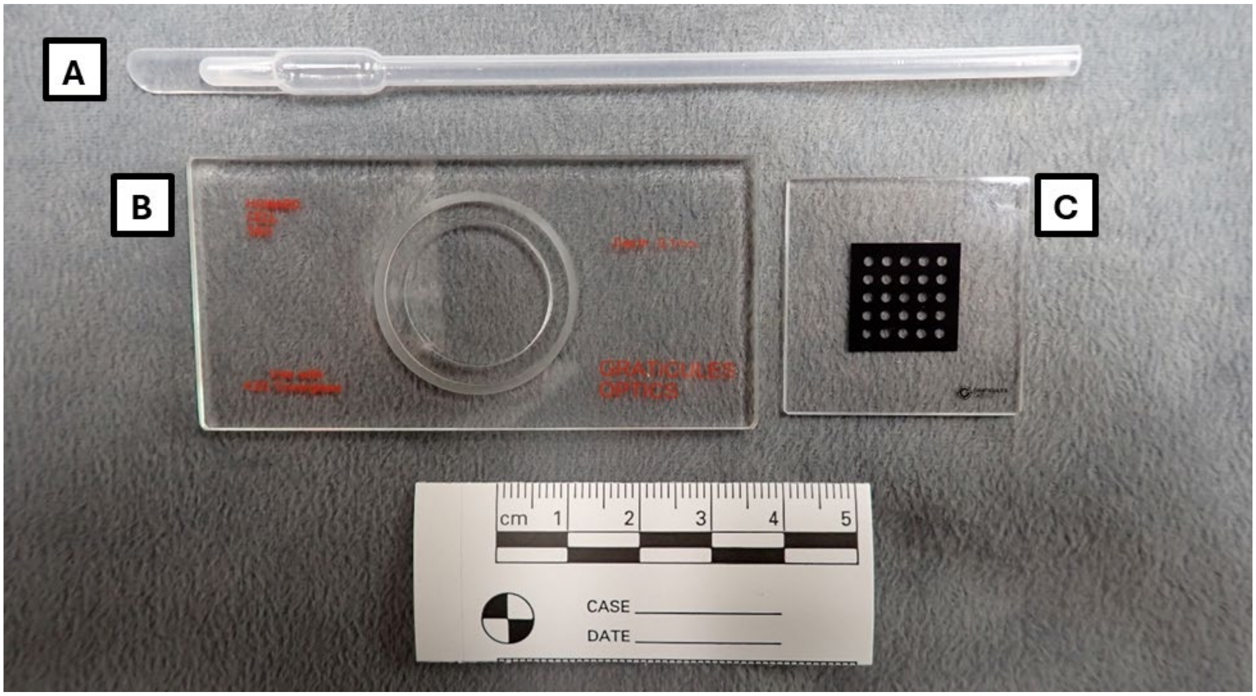

Kuvings EVO820 Whole slow juicer, with juicing strainer screen (0.5334 mm top & 0.4572 mm bottom). Depicted assembled (A), disassembled (B), and just the screen (C).

**Appendix 3**




Robot Coupe C80, with 0.5 mm screen. Depicted assembled (A), disassembled (B), and just the screen (C).

**Appendix 4**




(A): Thermo Scientific 295 Samco Special Purpose non-sterile Padl-Pet 50 µl drop size transfer pipette ( 13-711-46), Thermo Fisher Scientific; (B): Graticules Optics Limited S60 Howard Cell Counting Cell (02C00420), Graticules Optics Limited; (C): K20 Segewick Rafter Counting Chamber (02C00419).