Is that Straw-bacteria I See?

I. Introduction

Strawberries are one of the most widely consumed fruits in the United States. However, given their soft and porous surfaces, strawberries are incredibly vulnerable to many forms of bacterial contamination, underscoring the importance of thoroughly washing them. Our goal was to identify whether washing strawberries significantly reduces bacteria—and if so, which method is best—providing real-world guidance for safer consumption.

To quantify the amount of bacteria present after each washing treatment, we used a spectrophotometer to measure absorbance of bacteria in Luria Bertani (LB) broth solutions in which we incubated swabs of bacteria collected from the surface of 108 separate strawberries. According to the Beer-Lambert Law, absorbance is directly proportional to concentration, allowing us to quantify the effectiveness of each washing method by comparing mean absorbances and concentrations between strawberries washed differently after allowing their respective bacterial cultures to grow.

II. Question & Hypotheses

Our research question is: <u>Does the washing method (no water, cold water, or warm water) affect the mean bacteria concentration of strawberries measured by the mean absorbance of the bacterial cultures?</u>

H_0: There is **no difference** in the true mean absorbance of bacterial cultures among strawberries washed with no water, cold water, and warm water. ($\mu_N = \mu_C = \mu_W$)

H_A: There **is a difference** in the true mean absorbance of bacterial cultures among strawberries washed with no water, cold water, and warm water for **at least two** washing methods.

($\mu_N \neq \mu_C \neq \mu_W$)

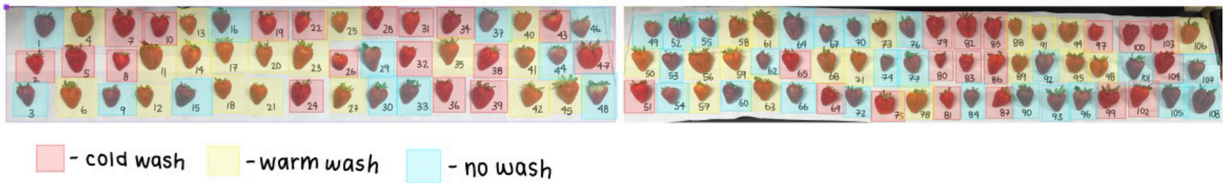
- $\mu_N =$ true mean absorbance of bacterial cultures among all strawberries like the ones in our study that are not washed.
- $\mu_C =$ true mean absorbance of bacterial cultures among all strawberries like the ones in our study that are washed with cold water.
- $\mu_W =$ true mean absorbance of bacterial cultures among all strawberries like the ones in our study that are washed with warm water.

III. Data Collection

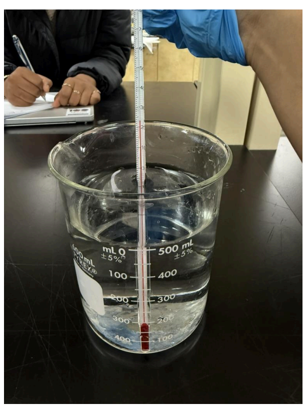
To conduct our experiment, we followed a rigorous methodology to ensure we controlled for potential confounding variables including temperature, washing method, and strawberry condition.

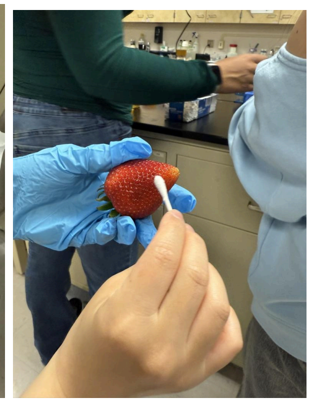
a) <u>Selecting Strawberries:</u> To gather our sample of strawberries, we used cluster random sampling so we could generalize our results to all non-organic strawberries sold at our local Trader Joe's in Highlands Pl, San Diego CA on May 11th, 2025, rather than only the ones similar to those in our experiment. After numbering all non-organic strawberry containers at the store from 1-40, we selected 3 unique integers using a random number generator, and used the strawberries in the corresponding boxes for our experiment.

b) <u>Randomly Assigning Treatments:</u> Before numbering our strawberries, we removed strawberries that were already showing mold. This ensured that all strawberries began the experiment in a similar condition, reducing the variability that could have been caused by existing bacteria or previous contamination, thus allowing us to attribute differences in bacteria concentration to the washing treatments. Since we had three treatments: no wash, cold wash, and warm wash, we used the randomization feature in Google Sheets to randomly assign 36 strawberries to each of the 3 treatments.

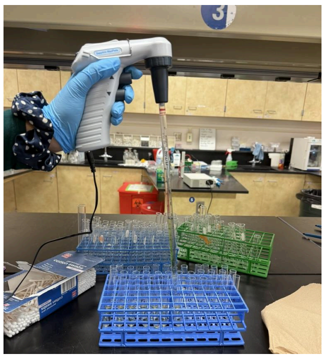
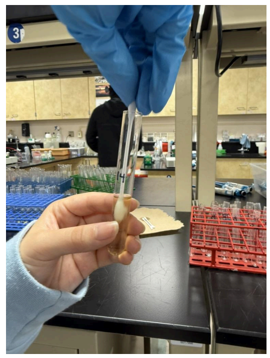
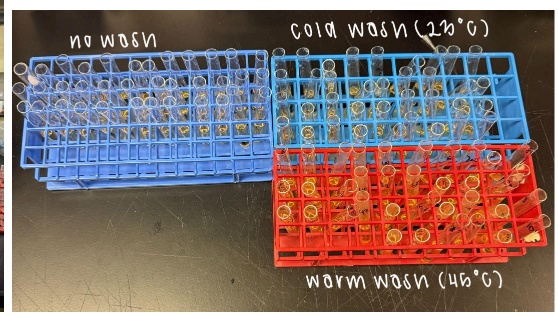


c) Washing Method: To control for variability in the way each strawberry was washed, the same washing steps were used for every strawberry, namely, soaking them in their respective temperatures for 30 seconds, then allowing them to dry in a paper towel. For the cold water group, we used water that was 23 degrees celsius, and for the warm water group, we used water that was 45 degrees celsius. For the no wash group, the strawberries were immediately swabbed.

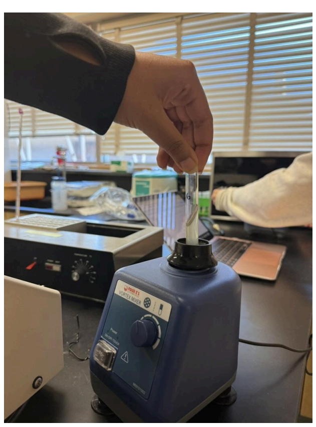




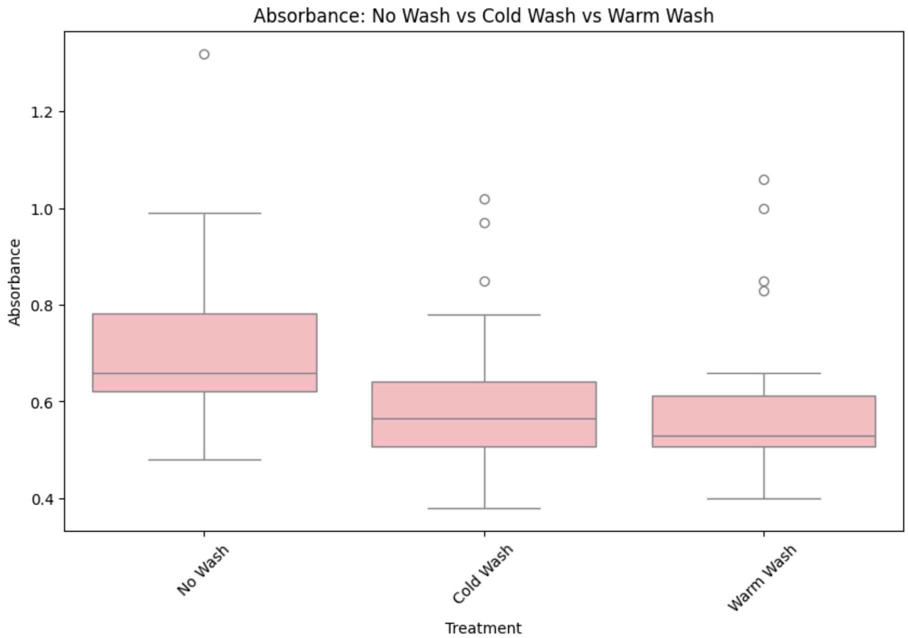
d) Swabbing Procedure: Prior to swabbing the strawberries, we prepared a nutrient broth, specifically a Luria Bertani broth, to act as a controlled environment for bacterial growth, providing necessary nutrients and growth factors for the bacteria to grow. To create the broth, we added 12.5 grams of the nutrient broth to 500 mL of deionized water (water void of any extra minerals or ions that may interfere with or cause chemical reactions). Then, we let it autoclave, or sterilize, for one 45 minute cycle to eliminate unwanted microorganisms that may contaminate or compete with the bacteria we wanted to grow. Equal volumes of the broth (4 mL) were pipetted into 90 sterile test tubes for measurement. After soaking in either warm or cold water for 30 seconds, each strawberry was swabbed using a q-tip by gently stroking the q-tip 30 times along all sides of the strawberry in the same direction. Then, we swirled the q-tips in each test tube 30 times to transfer the bacteria.

e) <u>Measurement:</u> After swabbing, we placed the tubes in an incubator at around 37 degrees celsius for two days, giving the bacteria a controlled environment where it could grow. After incubation, a Vortex device was used to mix the bacteria within the broth for each test tube. Then, the tubes (which were wiped down with a Kimwipe to remove fingerprints and dirt) were placed in a spectrophotometer, which tested the absorbance of the solutions at 550 nm light, which we could then use to determine the relative concentration of bacteria for each tube. The spectrophotometer was set to the same initial settings for each tube.




IV. Data Display



Summary Statistics:

	No Wash	Cold Wash	Warm Wash
Average absorbance	0.72	0.590	0.58
Standard deviation	0.170	0.140	0.146
Variance	0.0289	0.0196	0.0212

V. Analysis: ANOVA

Since concentration is directly proportional to absorbance, we noticed the mean concentration of bacteria was much lower in the cold and warm treatments compared to the no wash treatment, but that the differences in means between the warm and cold treatments were not as large. To determine whether these differences were significant, we conducted an **ANOVA Test** at a significance level of 0.05.

Conditions for ANOVA:

1) **Random:** This condition is met as we used random assignment to assign the strawberries to each of our three treatments: no wash, warm wash, and cold wash.
2) **Independence:** Since this was an experiment, the 10% condition does not apply. However, we can assume independence as each strawberry is independent of other strawberries within the same group.
3) **Normality:** Since each treatment group had $36 \geq 30$ strawberries, the Central Limit Theorem is met so we can assume the sampling distributions of mean bacteria for the no wash, cold wash, and warm wash groups are all approximately normal.
4) **Homogeneity of Variance:** The variances of the no wash, warm wash, and cold wash groups should be roughly equal. The lowest variance is .0196 for the cold wash group, and the largest variance is .0289 for the no wash group. Since the ratio of highest:lowest variances is 1.47<2, we can assume roughly equal variances.

Since all of the conditions were met, we ran an ANOVA test and created the following ANOVA table:

Source	Sum of Squares (SS)	Degrees of Freedom (df)	Mean Squares	F statistic	p-value
Treatment (between-treatments)	.408	2	MST: .204	8.78089496	0.000298
Error (within-treatments)	2.440	105	MSE: .023		
Total	2.849	107	.227		

F-critical: $F_{.05(2,\,105)} = 3.083$

VI. Conclusion: ANOVA

Our F-Statistic, which was calculated from dividing the MST by the MSE, was 8.78, indicating that the variance between our no wash, cold wash, and warm wash treatments was greater than the total variance within each individual treatment. When compared to our critical f-value of 3.083, we can see that an f-statistic of 8.78 will fall into the rejection region of the respective f-distribution. This also indicates that the variance between different groups cannot be fully explained by the MSE, providing evidence that there is a significant difference in means between the treatments.

Since our p-value (0.000298) for our ANOVA test is less than our significance level of 0.05, we can reject the null hypothesis. Therefore, we have convincing evidence that there is a difference between at least two of the true mean absorbance of bacterial cultures among strawberries washed with no water, cold water, and warm water, which is directly proportional with the concentration of bacteria in the cultures. If we assume that the null hypothesis is true (all the treatment groups have an equal true mean of absorbances), there is a 0.000298 probability that we get a F-statistic of 8.781 or greater; in other words, observing a difference in sample means as different or more different as what we have observed.

VII. Analysis: Post-Hoc Test

In addition to our ANOVA test, we conducted a Tukey Honestly Significant Difference test to determine which specific pairs of mean bacteria concentrations were significantly different from each other. Since all the conditions for the ANOVA test were met and each treatment's sample size was the same (36), we created the following Tukey HSD table:

Treatments	Difference in means	Tukey HSD Statistic	p-value	Significance
No wash - cold wash	.125	.085	0.00205	.00205<.05 → Significant
No wash - warm wash	.135	.085	0.00082	.00082<.05 → Significant

Cold wash - warm wash	.010	.085	0.96064	.96>.05 → Not Significant

$k = 3, df_{within} = 105, Q\ critical\!: Q_{.05(3,\,105)} = 3.36$

VIII. Conclusion: Post-Hoc Test

Because the HSD value of 0.085 was smaller than the differences in no wash - cold wash and no wash - warm wash, we can reject the null hypothesis. Therefore, we have convincing evidence that there is a statistically significant difference between the true mean concentrations of bacteria cultures for no wash compared to cold wash and no wash compared to warm wash at a significance level of 0.05. However, the p-value of .96 for the cold wash - warm wash treatments means that we fail to reject our null and do not have convincing evidence of a difference in mean concentration of bacteria for strawberries washed with cold versus warm water.

IX. Practical Significance

While our p-values from our ANOVA and Post-Hoc tests showed statistically significant differences in mean bacteria concentrations between the different washing treatments in our study, we also calculated the effect size of our results using Cohen's *d* coefficient, which helped us determine practical significance, namely, whether these differences are large enough to be meaningful in the real world. Using the following formula: $d = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{\frac{s_1^2 + s_2^2}{2}}}$, we calculated the following values:

$\lvert\bar{x}_1 - \bar{x}_2\rvert$	Pooled SD	Cohen's *d* coefficient

$\lvert \bar{x}_{no\ wash} - \bar{x}_{cold\ wash} \rvert = .125$	0.155724	0.80
$\lvert \bar{x}_{no\ wash} - \bar{x}_{warm\ wash} \rvert = .135$	0.158317	0.85
$\lvert \bar{x}_{warm\ wash} - \bar{x}_{cold\ wash} \rvert = .010$	0.142878	.07

With coefficients greater than or equal to .80 for the cold and warm water treatments when compared with the no water treatment, we can conclude that washing strawberries with both warm or cold water have large, practically significant effects on reducing the mean concentration of bacteria in strawberries. However, the low coefficient of .07 between warm and cold water indicates that there is very little meaningful difference between the effectiveness of cold and warm water in reducing mean bacteria concentration.

X. Reflection

Our project's goal was to determine whether washing methods have an effect on the mean concentration of bacteria in strawberries. One possible source of error we could've encountered was the inconsistency of the spectrophotometer we used to measure the relative concentrations of the test tubes. This could have resulted in the high outliers visible in our boxplots, which increase the variance of concentrations within each washing group, thus decreasing the f-statistic and making it more likely to falsely reject the null hypothesis. Additionally, we did not wash the inside of the test tubes prior to adding the nutrient broth, which could've also increased the variability in our results. If we were to have repeated our experiment, we would make sure to thoroughly clean each tube, so we would be able to reduce confounding related to the tubes' previous bacterial contamination. Considering we rejected our null hypothesis, there is a possibility that we made a Type I error, where we established that there is a difference between the mean absorbance levels among strawberries given the three different washing methods, when in reality there is no significant difference. Nevertheless, through controlling other confounding variables in our experiment, we are confident that our project provides convincing evidence that washing strawberries does have a significant effect on bacteria concentration.

XI. Appendix

Raw Data:

strawberry #	wash method	absorbance	strawberry #	wash method	absorbance	strawberry #	wash method	absorbance
1	no wash	0.74	2	cold wash	0.43	4	warm wash	0.85
3	no wash	0.63	5	cold wash	0.41	6	warm wash	0.48
9	no wash	0.62	7	cold wash	0.85	11	warm wash	0.47
15	no wash	0.62	8	cold wash	0.66	12	warm wash	0.52
16	no wash	0.57	10	cold wash	0.7	13	warm wash	0.83
29	no wash	0.61	19	cold wash	0.64	14	warm wash	0.49
30	no wash	0.97	22	cold wash	0.38	17	warm wash	0.47
33	no wash	0.63	24	cold wash	0.97	18	warm wash	0.53
37	no wash	0.62	26	cold wash	0.78	20	warm wash	0.53
44	no wash	0.63	28	cold wash	0.55	21	warm wash	0.63
46	no wash	0.93	31	cold wash	0.57	23	warm wash	0.52
48	no wash	0.6	32	cold wash	0.47	25	warm wash	0.51
49	no wash	0.98	34	cold wash	0.56	27	warm wash	0.53
52	no wash	0.79	36	cold wash	0.5	35	warm wash	0.64
53	no wash	0.78	38	cold wash	0.53	40	warm wash	0.4
54	no wash	0.67	39	cold wash	0.54	41	warm wash	0.51
55	no wash	0.75	43	cold wash	0.59	42	warm wash	0.64
60	no wash	0.99	47	cold wash	0.54	45	warm wash	0.57
62	no wash	0.5	51	cold wash	0.64	50	warm wash	0.55
64	no wash	0.85	65	cold wash	0.53	56	warm wash	0.56
66	no wash	0.62	69	cold wash	0.5	57	warm wash	0.53
67	no wash	0.68	75	cold wash	0.6	58	warm wash	0.53
70	no wash	0.48	79	cold wash	0.57	59	warm wash	0.5
72	no wash	0.64	80	cold wash	0.55	61	warm wash	0.62
74	no wash	0.73	81	cold wash	0.47	63	warm wash	0.42
76	no wash	0.79	82	cold wash	0.62	68	warm wash	1.06
77	no wash	0.73	83	cold wash	0.41	71	warm wash	0.53
84	no wash	0.5	85	cold wash	0.65	73	warm wash	1
90	no wash	0.56	86	cold wash	0.51	78	warm wash	0.5
92	no wash	0.6	87	cold wash	0.58	88	warm wash	0.55
93	no wash	1.32	97	cold wash	0.51	89	warm wash	0.61
96	no wash	0.93	99	cold wash	1.02	91	warm wash	0.4
101	no wash	0.66	100	cold wash	0.63	94	warm wash	0.61
105	no wash	0.66	102	cold wash	0.61	95	warm wash	0.6
107	no wash	0.66	103	cold wash	0.5	98	warm wash	0.54
108	no wash	0.71	104	cold wash	0.67	106	warm wash	0.66
avg absorbance		0.7153			0.5900			0.5803
stdev absorbance		0.1700			0.1400			0.1457
variance		0.0289			0.0196			0.0212

ANOVA Calculations:

calculating SSt			
sample mean	0.715	0.590	0.580
grand mean:	0.629	0.629	0.629
n(xibar-GM)^2	0.271	0.053	0.084
SSt=		0.408	

	calculating SSe		
	No wash	cold wash	warm wash
(xi-xibar)^2	0.000611	0.025600	0.072750
	0.007272	0.032400	0.010056
	0.009078	0.067600	0.012161
	0.009078	0.004900	0.003633
	0.021106	0.012100	0.062361
	0.011083	0.002500	0.008150
	0.064883	0.044100	0.012161
	0.007272	0.144400	0.002528
	0.009078	0.036100	0.002528
	0.007272	0.001600	0.002472
	0.046106	0.000400	0.003633
	0.013289	0.014400	0.004939
	0.070078	0.000900	0.002528
	0.005583	0.008100	0.003567
	0.004189	0.003600	0.032500
	0.002050	0.002500	0.004939
	0.001206	0.000000	0.003567

	0.075472	0.002500	0.000106
	0.046345	0.002500	0.000917
	0.018150	0.003600	0.000411
	0.009078	0.008100	0.002528
	0.001245	0.000100	0.002528
	0.055356	0.000400	0.006445
	0.005667	0.001600	0.001578
	0.000217	0.014400	0.025689
	0.005583	0.000900	0.230133
	0.000217	0.032400	0.002528
	0.046345	0.003600	0.176167
	0.024111	0.006400	0.006445
	0.013289	0.000100	0.000917
	0.365689	0.006400	0.000883
	0.046106	0.184900	0.032500
	0.003056	0.001600	0.000883
	0.003056	0.000400	0.000389
	0.003056	0.008100	0.001622
	0.000028	0.006400	0.006356
SSe = sum of all (xi-xibar)^2 =		2.440394	