Detection of Microbiological Contamination in Selected Vegetables and Prevention of Foodborne Diseases

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BIOLOGY EXTENDED ESSAY

Research Question: ***How do the number of colonies of Escherichia coli in Petroselinum crispum and Lactuca sativa samples are changed, when samples are soaked into 0 ppm, 1,000 ppm, 1,500 ppm, 2,000 ppm and 2,500 ppm concentrations of acetic acid (in vinegar) for 10 minutes during cleaning process and inoculated later on violet red bile (VRB) agar?***

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I. INTRODUCTION

Fresh produce is an essential food that provides wide variety of nutrients and fiber to health-conscious consumers (Carlin, F., 2007). It includes vegetables, fruits, seeds, herbs and nuts.

Fresh produce may cause foodborne diseases from harmful microorganisms such as *Listeria*, *Coliforms*, and *Salmonella.* Fresh produces like fruits and vegetables can be contaminated in the long food chain reaching the consumers (Centers For Disease Control and Prevention, 2020).

In global terms, every year, almost 600 million people – nearly one out of every ten people on the planet – become infected after consuming contaminated food and 420,000 of them die. Unsafe food costs US$110 billion to low- and middle-income countries due to loss in production and medical expenses (World Health Organization (WHO), 2021). As a result of epidemiological studies, a substantial portion of recent foodborne outbreaks has been linked to the consumption of infected fresh produce with pathogens all over the world (Gorny, J., 2006).

Among others, coliform bacteria are referred as indicator organisms because their presence in food suggests that the conditions are favorable for the presence of enteric pathogens and may refer to unsatisfactory sanitary exercise.

Coliform bacteria generally includes four species of enterobacteriaceae: *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Escherichia coli*. Some coliform bacteria are affiliated with the intestines of warm-blooded animals, called fecal coliforms. Various of them like *Erwinia* and *Enterobacter*, are part of the natural flora of many vegetables and generally may not suggest a potential health problem (Batt,

Carl A. *et al*, 2014). However, the high total coliform count may refer to a health risk in vegetables and needs further attention in laboratory detection (Lahukar M. B. *et al*, 2017).

The aim of this experiment is to observe the existence of a harmful coliform *Escherichia coli*, that spreads and can be detected easily, as a source of an evidence for cause of a likely foodborne disease in two green leafy vegetables *Lactuca sativa (lettuce)* and *Petroselinum crispum (parsley)* and treatment of these vegetables with different concentrations of acetic acid (vinegar) during washing them to clean in order to assess the impact of this treatment on reducing the number of coliform bacteria and specifically number of *E.coli*.

The reason that I have selected *Lactuca sativa* and *Petroselinum crispum* in my experiment is that these crops are regularly irrigated during growth. As they are produced in the areas in the neighborhood of big cities, the irrigation water is polluted most of the times. The intake of these freshly consumed leafy green vegetables by humans may cause serious diseases if necessary precautions are not considered (Aytaç, S.A., 2010).

I set my **research question** as; how do the number of colonies of *E.coli* in *P.crispum* and *L.sativa* samples are changed, when samples are soaked into 0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm concentrations of acetic acid (in vinegar) for 10 minutes during cleaning process and inoculated later on violet red bile (VRB) agar?

II. HYPOTHESIS

In Turkey green edible leaves (*Lactuca sativa, Petroselinum crispum*) are traditionally washed in a vinegar (5% acetic acid in volume) containing cup with tap water in order to kill germs and to have a safer food. As these leaves are not cooked before consumption the hygiene of these foods are important in order not to have food-borne diseases due to any contamination on these raw food materials. Therefore the hypothesis is increasing concentrations of acetic acid

(vinegar) in washing process decrease the presence of *E.coli* in *Lactuca sativa (lettuce),* and *Petroselinum crispum (parsley)* (Temiz, A. *et al*, 2011) samples.

III. VARIABLES AND MATERIALS

III.1. Variables

Independent variables	*L.sativa* and *P.crispum* in tap water, and in different concentrations of acetic acid (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm).
Dependent variable	Number of bacterial colony on VRB agar
Controlled variables	Bacteria inoculation temperature being 37°C, dilutions of 1/10 or 1/100 of plant samples, incubation time (30 min.), volume of powder (500 g) agar used in preparation of type of agar used (VRB), mass of *L.sativa* and *P.crispum* (25 g each)

Table-1: Various variables used in the experiment

III.2. Materials List

Item	Quantity	Specification
- *Lactuca sativa*	1 piece	
- *Petroselinum crispum*	1 piece	
- *Vinegar*	1 bottle, 500 ml	
- Washing bowl	5 pieces	
- Petri dishes	6 x 5 x 2 = 60 pieces	For two crops
- Agar powder	2 bottle VRB (violet red bile agar)	500 g
- Dilution glass bottles	10 pieces	
- Microwave oven	1	
- NaCl	1 bottle	50 g
- Incubator	1	
- Refrigerator	1	
- Stomacher	1	
- Pipets with pipet aids	5 pieces	
- Circulating water bath, for tempering agar, thermostatically controlled	1	
- Thermometer	1	Mercury
- Bunsen burner	1	
- Plastic packs	10 Pieces	

Table-2: Materials used in the experiment

IV. METHOD DEVELOPMENT

IV.1. Preparation of Samples

To analyze any microbiological effect on any food product, there is a need for a scientific food laboratory with necessary equipment and substances. I have visited and explained my experiment at the Food Engineering Department of Hacettepe University. Thanks to their understanding, I provided Department's permission to conduct experiments in the microbiology laboratory. I have planned and completed my experiment in July 2021.

L.sativa and *P.crispum* and a bottle of vinegar were purchased from the local market place for the analysis. Both plants were put and waited in five different solutions for 10 minutes to be processed later for inoculation: Tap water without treating with acetic acid (to see the impact of washing the plants with tap water for cleaning), and in different concentrations [40 ml vinegar (2 ml acetic acid) with 2 liters H_2O (1000 ppm), 50 ml vinegar (2.5 ml acetic acid) with 1.67 liters H_2O (1500 ppm), 50 ml vinegar (2.5 ml acetic acid) with 1.25 liters H_2O (2000 ppm) and 50 ml vinegar (2.5 ml acetic acid) with 1 liter H_2O (2500 ppm)] of acetic acid in vinegar with 5% acetic acid in volume. 25 grams of each vegetable sample were prepared and placed in a stomacher bag in 225 ml of distilled water. These samples for each vegetable were placed in the refrigerator for further use. The given concentrations were chosen to imitate the behaviour of consumers using little concentrations of vinegar in cleaning vegetables before consumption. Different serial dilutions were used to process samples as the number of expected microorganisms would be very high to count on the plates. As it is a usual practice in many of experiments in food analysis to be discussed further in this study, different dilutions of 1/10, 1/100 were prepared from the stomacher bag content for *L.sativa* and *P.crispum* respectively, and were inoculated in the trials in VRB media. The *E.coli* concentration in the *P.crispum* samples has been found higher in another study in the literature due to the processing of this plant after harvest and during packaging with a greater surface to be easily contaminated before

consumption (Aycicek, H. *et al*, 2006). This finding referred to the need of more diluted samples in my experiment. The number of colonies for the samples of *P.crispum* were expected to be very high in the sample dilution of 1/10, therefore, more diluted sample of 1/100 was used where the total colonies were more countable on the plates. For *L.sativa* the sample dilution of 1/10 was used as the number of colonies could be easily differentiated during the analysis.

Therefore 6 trials were strived for each line of samples and sum up to 30 trials in the media. Therefore, totally 30 trials were performed for one plant sample and 60 trials as a gross total of experiment.

One of the concerns during the trials was the time constraint for inoculation of samples and complete all trials of samples of plants in one day for each. Experiment was conducted in two days in each plant sample including the preparation, implementation and reporting. I purchased the chemicals used in the experiment from dealers in the market.

IV.2. Preparation of Agar

L.sativa and *P.crispum* were processed for inoculation to see the impact of washing the plants with tap water for cleaning, and in different concentrations of acetic acid in vinegar (0, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm). Violet red bile agar is one of the ideal media for counting coliform bacteria and can specifically be used for the growth of *E.coli* (Venkateswaran, K. *et al*, 1996).

VRB agar is prepared by suspending 38.5 g of agar powder in 1 litre of distilled water and it is heated to boiling with stirring until completely dissolved. Sterilization is completed during the melting process. The content of agar VRB is meat-peptone lactose, yeast extract with bile salt mixture agar. The content of agar permits the growth of coliform bacteria and E.coli for our analysis.

IV.3. Pour Plate Technique

1/10 and 1/100 dilutions of samples were used for *L.sativa* and *P.crispum* respectively to inoculate in VRB agar to count *E.coli* colonies. Microbial populations exist with a mixture of many other cell types and these populations can be separated into pure cultures. In order to determine viable cells the serial dilution–agar plate technique is used. Using VRB agar to isolate *E.coli* colonies in *L.sativa* and *P.crispum*, pour plate procedure (Aryal, S., 2019-2) was conducted during the experiment.

IV.4. Method

1. Prepare different concentrations of vinegar with 5% acetic acid in volume. This means in 500 ml bottle of vinegar there is 25 ml acetic acid. To prepare different concentrations of acetic acid content, mix 40 ml vinegar (2 ml acetic acid) with 2 liters H_2O (1000 ppm), 50 ml vinegar (2.5 ml acetic acid) with 1.67 liters H_2O (1500 ppm), 50 ml vinegar (2.5 ml acetic acid) with 1.25 liters H_2O (2000 ppm) and 50 ml vinegar (2.5 ml acetic acid) with 1 liter H_2O (2500 ppm) measured in dilution bottles and pour them seperately into prepared basins with labeling each. The sample with 0 ppm was also labelled.
2. Take 25 grams of *L.sativa* sample and put it into 225 ml of 0 ppm labelled solution containing stomacher bag and wait for 10 minutes to be processed later for inoculation.
3. Put the bag into stomacher and wait for 5 minutes in order to have them squished.
4. Prepare 1/10 dilution of 0 ppm solution of *L.sativa* (Figure-1)
5. Repeat steps from 2 to 4 for solutions of 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm of *L.sativa*.
6. Repeat steps from 2 to 5 for *P.crispum* but with 1/100 dilution of solutions.

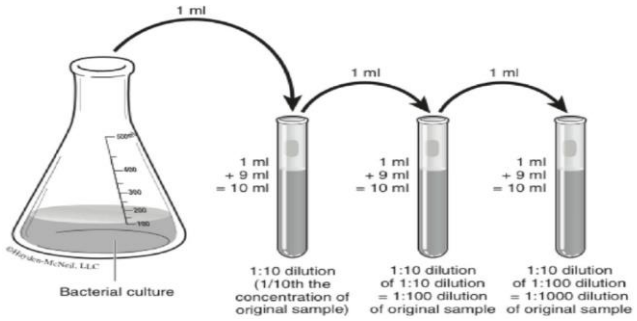


Figure-1: Dilution of bacterial culture (Hester, L. *et al*, 2014)

VRB Agar:

7. Prepare 1 lt volume of pre-sterilized VRB agar for the experiment (see section IV.2 for preparation of VRB agar). Ten minutes before pouring plates, melted VRB agar is transferred from the 55 °C water bath to a heat block on the laboratory bench set at 48 °C. Once the agar reaches this optimum temperature, it is ready to pour.
8. Label petri dishes as 0 ppm of 1/10 dilution of *L.sativa.*
9. Pipette out samples of 1 ml from 1/10 dilution of *L.sativa* samples into the middle of the petri dishes after opening their lids under sterile conditions and close the lids.
10. Remove the cap of the tube of the melted agar and under sterile conditions pour 35 ml of agar carefully into the petri dish containing the sample.
11. Close the lid of the plate and mix the sample gently with the agar by swirling the plate.
12. Leave the agar to solidify before inverting the plate for incubation at 37°C for 24 hours.
13. Repeat steps 8 to 12 for solutions of 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm of *L. Sativa*.
14. Repeat steps from 8 to 13 for *P.crispum* but with 1/100 dilution of solutions.
15. Repeat steps 1-14 for 5 more times to have 6 trials.
16. Count the colonies and do statistical analyses.

V. DATA COLLECTION AND PROCESSING

Among different volumes of acetic acid, I performed 6 trials for each as a sum of repetition trials. Among dilutions the colonies were counted for each sample. Therefore, results of these 6 trials on each concentration of acetic acid (total of 30 records for each plant sample and total of 60 in the media) seem sufficient to observe the impact of increasing volumes of acetic acid on number of growing colonies.

V.1. Data Collection

The results of the analyses for each sample are given below concerning total bacteria colonies in each sample.

Petroselinum crispum (parsley)	Trial	Number of *E.coli* Colonies				
		0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
	1	48	42	37	30	11
	2	49	40	34	27	15
	3	52	45	38	28	17
	4	50	38	33	29	10
	5	49	36	34	22	12
	6	53	40	32	31	15

Table-3: Raw Data of Trials, Number of Colonies for *Petroselinum Crispum* on VRB among different Acetic Acid concentrations (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm)

Lactuca sativa (lettuce)	Trial	Number of *E.coli* Colonies				
		0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
	1	37	33	26	22	16
	2	42	32	27	21	12
	3	39	34	24	24	17
	4	43	30	28	16	19
	5	41	32	32	24	16
	6	40	33	25	25	20

Table-4: Raw Data of Trials, Number of Colonies for *Lactuca sativa* on VRB among different Acetic Acid concentrations (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm)

V.2. Calculation of total number of bacteria among colonies in the samples

The total number of colony counts has to be transformed to colony forming units (CFU)/ml. CFU is a unit used in microbiology to estimate the number of bacteria in a sample that has the ability to multiply under the controlled conditions. Counting with colony-forming units requires culturing the microorganisms and counts viable cells. A colony that clearly appears on the plate has a significant growth, but it is uncertain if the colony grew from one cell or a group of cells. Therefore, it is required to express the count in colony-forming units to reflect this uncertainty (Stewart, L., 2021).

Here I concentrate on the calculation of CFU per ml of *E.coli* in VRB agar. For the count, test results divided by the dilution factor that is the volume of plant sample used for inoculation on agar multiplied by the sample dilution used in the test. I have to note that I have 6 records (total of 30) among acetic acid concentrations for each plant. The calculations were conducted for 1/10 dilution of *L.sativa* and 1/100 dilution of *P.crispum*.

As the pour plate technique is used in VRB to count colonies of *E.coli*, the formula is given below for this technique. In the count of microorganisms, one can choose to calculate either averages of parallel trials or only one of the trial.

$$N = \frac{(\text{No.of microorg. on plate 1 of sample of plant i} + \text{no.of microorg. on plate 2 of sample of plant i}) \ldots}{\text{volume of sample on the plate (1 ml)} \; x \; (number\ of\ plates) \; \text{x dilution used in the test}} \quad (1)$$

In the pour plate example; the number of colonies on VRB agar in 1000 ppm acetic acid for *P.crispum* using sample dilution of 1/100 were counted as 42 in the first trial (Table-3):

$N = (42) / (1x1x0.01) = 4.2 \times 10^3$ CFU/ml

Petroselinum crispum (parsley)	Trial	Colony forming units - CFU/ml *E.coli*				
		0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
	1	4 800	4 200	3 700	3 000	1 100
	2	4 900	4 000	3 400	2 700	1 500
	3	5 200	4 500	3 800	2 800	1 700
	4	5 000	3 800	3 300	2 900	1 000
	5	4 900	3 600	3 400	2 200	1 200
	6	5 300	4 000	3 200	3 100	1 500

Table-5: Total Number of *E.coli* in *Petroselinum crispum* Samples in different Acetic Acid contents (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm)

Lactuca sativa(lettuce)	Trial	Colony forming units - CFU/ml of *E.coli*				
		0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
	1	370	330	260	220	160
	2	420	320	270	210	120
	3	390	340	240	240	170
	4	430	300	280	160	190
	5	410	320	250	240	160
	6	400	330	320	250	200

Table-6: Total Number of *E.coli* in *Lactuca Sativa* Samples in different Acetic Acid contents (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm)

The figures in the tables above were calculated using formula (2) and recorded accordingly.

V.3. Statistical Calculations

1) Mean of total number of coliform bacteria

$$\bar{x} = \frac{\sum x}{n} \qquad (2)$$

In equation (2) **n** is the number of trials in a certain group of plant sample,

x is the number of coliform bacteria counted in different trials.

Example: For *Petroselinum crispum* sample, the mean of total CFU/ml of *E.coli* bacteria on the petri dishes using VRB in 2500 ppm acetic acid concentration where $\bar{\mathbf{x}}$ is 1333.3 from Table 5, can be calculated as:

$$\bar{x} = \frac{1100 + 1500 + 1700 + 1000 + 1200 + 1500}{6} = 1333.3$$

2) Standard Deviation of total number of coliform bacteria

$$s = \sqrt{\frac{\sum(X - \bar{x})^2}{n - 1}} \quad (3)$$

Equation (3) is used in the calculation of standard deviation;

n is the number of the trials in a certain group of plant sample,

x is the number of coliform bacteria counted in different trials,

$\bar{\mathbf{x}}$ is the mean of the number of coliform bacteria counted in different trials.

Example: For *P.crispum* sample, standard deviation of total CFU/ml of *E.coli* bacteria on the petri dishes using VRB in 2500 ppm acetic acid concentration where $\bar{\mathbf{x}}$ is 1333.3 from Table 5, can be calculated as:

$$s = \sqrt{\frac{(1100 - 1333.3)^2 + (1500 - 1333.3)^2 + (1700 - 1333.3)^2 + (1000 - 1333.3)^2 + (1200 - 1333.3)^2 + (1500 - 1333.3)^2}{5}}$$

$$= 273.25$$

Petroselinum Crispum (parsley)	Item	0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
	Mean of no.of *E.coli* in CFU/ml	5 016.7	4 016.7	3 466.7	2 783.3	1 333.3
	St. Dev.	194.08	312.52	233.81	318.85	273.25

Table-7: Mean and standard deviation of *E.coli* in *Petroselinum crispum* Samples (CFU/ml)

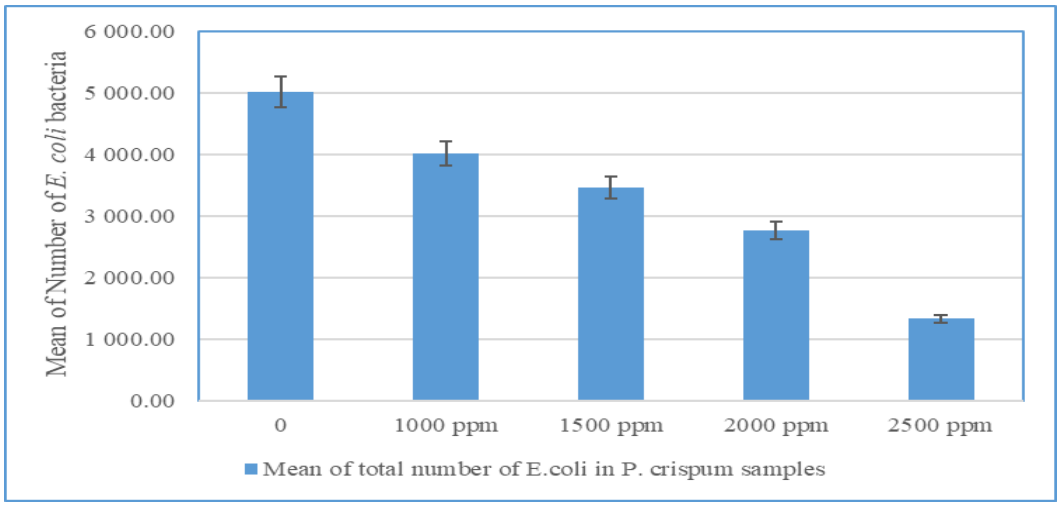
	Item	0 ppm acetic acid	1000 ppm acetic acid	1500 ppm acetic acid	2000 ppm acetic acid	2500 ppm acetic acid
Lactuca sativa(lettuce)	Mean of no.of *E.coli* in CFU/ml	403.3	323.3	270.0	220.0	166.7
	St. Dev.	21.60	13.66	28.28	32.86	28.05

Table-8: Mean and standard deviation of *E.coli* in *Lactuca sativa* Samples (CFU/ml)

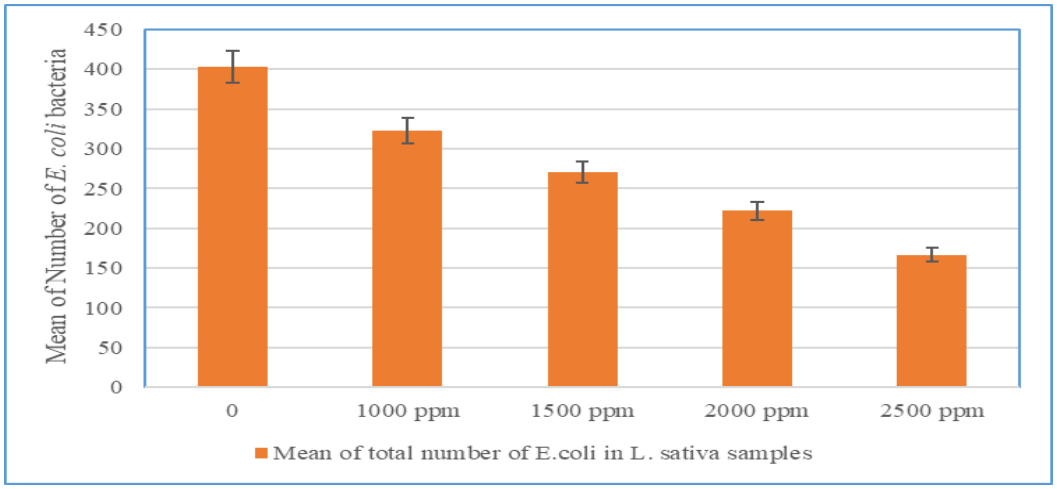
V.4. Graphs

Concentrations of acetic acid	Mean of total number of *E.coli* (CFU/ml) in *P. crispum* samples	Mean of total number of *E.coli* (CFU/ml) in *L. sativa* samples
0	5 016.7	403.3
1000 ppm	4 016.7	323.3
1500 ppm	3 466.7	270.0
2000 ppm	2 783.3	220.0
2500 ppm	1 333.3	166.7

Table-9: Means of Total number of *E.coli* (CFU/ml)



Graph-1: Mean of Number of *E.coli* (CFU/ml) Bacteria in *Petroselinum crispum* Samples in VRB media among different concentrations of acetic acid



Graph-2: Mean of Number of *E.coli* (CFU/ml) Bacteria in *Lactuca sativa* Samples in VRB media among different concentrations of acetic acid

V.5. ANOVA Tests

Analysis of variance, or ANOVA, is a statistical method that separates observed variance data into different components to use for additional tests. A one-way ANOVA is used for three or more groups of data, to gain information about the relationship between the dependent and independent variables. In order to run ANOVA tests a null hypothesis has to be determined regarding my hypothesis for the experiment.

H_0: There is no statistical difference between the effects of different acetic acid concentrations on total number of *E.coli* colonies in *P.crispum/L.sativa* samples.

H_1: There is statistical difference between the effects of different acetic acid concentrations on total number of *E.coli* colonies in *P.crispum/L.sativa* samples.

Therefore considering the number of *E.coli* colonies in the samples calculated in the previous section, the ANOVA tests can be run using the features in Excel.

ANOVA for *Petroselinum crispum* (parsley) samples among changing acetic acid concentration

Group	*Trials*	*Total*	*Mean*
1	6	301	50.16667
2	6	241	40.16667
3	6	208	34.66667
4	6	166	27.66667
5	6	80	13.33333

Source of Variance	*Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Between Groups	4583.133	4	1145.783	161.2265	1.84E-17
Within Groups	177.6667	25	7.106667		
Total	4760.8	29			

Table-10: Results of ANOVA Analysis for *P.crispum*

ANOVA *Lactuca sativa* (lettuce) samples among changing acetic acid concentration

Group	*Trials*	*Total*	*Mean*
1	6	242	40.33333
2	6	194	32.33333
3	6	162	27.00000
4	6	133	22.16667
5	6	100	16.66667

Source of Variance	*Sum of Squares*	*df*	*Mean Square*	*F*	*P-value*
Between Groups	2003.467	4	500.8667	73.29756	1.94E-13
Within Groups	170.8333	25	6.833333		
Total	2174.3	29			

Table-11: Results of ANOVA Analysis for *L.sativa*

It is detected from the ANOVA test results checking the P-value (Bobbitt, Z., 2021) that in both of the samples, P-values at 5% significance level, are small ($1.84.10^{-17}$ for *P.crispum* and $1.94.10^{-13}$ for *L.sativa*). Therefore we have to reject H_0 and accept H_1, this means we accept

the difference between the effects of different acetic acid concentrations on total number of *E.coli* colonies in *P.crispum* and *L.sativa* samples in separate tests. There is significant difference among different concentrations of acetic acid applied to different plant samples in the experiment.

The displayed ANOVA results do not identify which particular differences between means of the trial pairs are significant. Therefore, I use post hoc (Tukey's) tests to analyze the differences among means of group pairs as I have same number of trials in each pair (Anonymous, 2022). Excel program has not that feature for analyses. Therefore I found the results after making calculations. Details are given below:

Pairs of groups	Difference of *means* of groups given in Table-10	*n1* (number of trials in 1st group)	*n2* (number of trials in 2nd group)	Standard Error *Sqrt(0.5*mean square*(1/n1+1/n2)*	q-value (Diff. of *means*/ St. Error)
1-2	10.00	6.00	6.00	1.09	9.19
1-3	15.50	6.00	6.00	1.09	14.24
1-4	22.50	6.00	6.00	1.09	20.67
1-5	36.83	6.00	6.00	1.09	33.84
2-3	5.50	6.00	6.00	1.09	5.05
2-4	12.50	6.00	6.00	1.09	11.49
2-5	26.83	6.00	6.00	1.09	24.66
3-4	7.00	6.00	6.00	1.09	6.43
3-5	21.33	6.00	6.00	1.09	19.60
4-5	14.33	6.00	6.00	1.09	13.17
Critical q-stat for df = 25 and k= 5 is 4.153, α=0.05					

Table-12: All pairwise group comparisons for *P.crispum* at alpha=0.05

Pairs of groups	Difference of *means* of groups given in Table-11	*n1* (number of trials in 1st group)	*n2* (number of trials in 2nd group)	Standard Error *Sqrt(0.5*mean square*(1/n1+1/n2)*	q-value (Diff. of *means*/ St. Error)
1-2	8.00	6.00	6.00	1.07	7.50
1-3	13.33	6.00	6.00	1.07	12.49
1-4	18.17	6.00	6.00	1.07	17.02
1-5	23.67	6.00	6.00	1.07	22.18
2-3	5.33	6.00	6.00	1.07	5.00
2-4	10.17	6.00	6.00	1.07	9.53
2-5	15.67	6.00	6.00	1.07	14.68
3-4	4.83	6.00	6.00	1.07	4.53
3-5	10.33	6.00	6.00	1.07	9.68
4-5	5.50	6.00	6.00	1.07	5.15
Critical q-stat for df = 25 and k= 5 is 4.153, α=0.05					

Table-13: All pairwise group comparisons for *L.sativa* alpha=0.05

For all pairs the *q-values* refer to a figure that is higher than the critical value given in the studentized range statistics (*q*) of 4.153 for a group of 5 and degree of freedom (df) of 25 at 5% significance level. All *q-values* among group pairs are over the critical value and all differences among means of group pairs are referred to be significant.

VI. ANALYSIS

In the experiment, *E.coli* count ranges of all performed trials for *P.crispum* samples were 6-15 times higher than the ones of *L.Sativa.* Therefore, *P.crispum* samples were observed to be more contaminated than *L.Sativa* samples. As I target to diminish the impact of microorganisms on the samples and used different concentrations of acetic acid, I observed that acetic acid could reduce the microbial load in the samples. From 0 (tap water) to a concentration of 2500 ppm of acetic acid, *E.coli* count were reduced at some extent but the level of microbial counts were still high at the maximum level of acetic acid treatment: CFU of *E.coli*

microorganisms in the samples were at about 1,350 and 170 on the average for *P.crispum* and *L.Sativa* respectively. This means a very high acetic acid concentration is needed to eleminate the microbial load in fresh produces.

The results obtained in this study showed that the levels of microbial contamination of the two leafy vegetables were high when bought from the open market, and the used concentrations of acetic acid had limited effect on reducing microorganisms.

The standard deviations of the data are generally diverse (changing between 5-20 % of the mean data) considering the whole set of data among concentrations and plant crops. When the standard deviation is small, then it means the results are close to the mean, but if the standard deviation is large, then the results are more spread out (Anonymous, 2021). This seems as a matter of non-standard growth of microorganisms in the media. Fluctuations are high as trials are related to a reproducing organism. But, the trends in each analysis refer to diminishing number of coliform bacteria when the acetic acid concentration is risen step by step. More visually the trend of diminishing number of coliform bacteria with the application of higher concentrations of acetic acid can be observed in graphs 1 & 2. As the acetic acid concentration increased the total number of *E.coli* bacteria calculated from these counts reduced.

The ANOVA analysis also refers to small P-value for both tests, at 5% significance level. H_0 is rejected and H_1 is accepted which means that we accept the difference between the effects of different acetic acid concentrations on total number of *E.coli* colonies are significant in both of the selected plant samples. Hence, a further Tukey analysis showed that the differences among means of group pairs were significant.

VII. EVALUATION

Strengths	Reason it is believed to be a strength
Appropriate statistical analysis	With a total of 6 trials in each set of acetic acid concentrations for two plant samples growing in VRB media, it is clear that the precision and accuracy of the results are reliable with ANOVA and post hoc tests.
Time of data collection	The experiment is conducted in 4 days, two days for each plant lots and data were collected in a short while without losing data quality.
Medium Conditions	A food laboratory located in one of the specialized university in Turkey is used for the experiment.
Use of Different Dilutions for the two plant samples	Bacterial growth in different sample dilutions ensures the growth of microorganisms to be detected and counted without taking a risk of failure in each trial.

Weaknesses	**Reason it is believed to be a weakness**	**Suggested improvement**
Bacterial Colony Count	*E.coli* bacteria are counted on agar with many samples in a short period, simple counting errors occur unintentionally.	Colonies to be counted under microscope.
Concentrations of acetic acid	Less number of concentrations to detect the decrease in number of bacteria.	Number of acetic acid concentrations to be increased with more resources.
Deficiency in Pour plate technique	The use of hot agar killing some contaminants to give a low count.	Inoculation to be performed at the right temperature using thermometer.
To overlook small colonies in pour plate technique	After incubation it is difficult to count number of small E.coli colonies.	A more accurate count can be maintained with use of microscope.

VIII. CONCLUSION

I set my **research question** as; how do the number of colonies of *E.coli* in *P.crispum* and *L.sativa* samples are changed, when samples are soaked into 0 ppm, 1,000 ppm, 1,500 ppm, 2,000 ppm and 2,500 ppm concentrations of acetic acid (in vinegar) for 10 minutes during cleaning process and inoculated later on violet red bile (VRB) agar?

The results of the experiment refer that the vinegar use at home kitchens aiming at eliminating the microbial causes of foodborne diseases would fail if people would only count on this technique. Consequently, this study displays the potential hazards of raw eaten salad vegetables. These plant crops may be contaminated by low quality irrigation water or through soil contamination. These results emphasize the importance of cleaning before raw consumption. Impractically high volumes of acetic acid may kill the germs in the leavy vegetables in daily use. However, consumers may not prefer to do so because it would be so costly but rather find alternative ways.

Public awareness has to be raised at all steps of the production, transport and consumption to reduce risks for consumers. The traditional ways of preventing contamination or foodborne diseases has to be discussed seriously in the public and alternative techniques have to be determined by the experts to combat with diseases originating from foods.

Sanitation of food via organic materials like acetic acid does not form any health risks for human beings. Therefore, in order to have safer food, the referred sanitation will be done via use of vinegar. At high acetic acid concentrations like 12,500 ppm (approximately 1 part vinegar in 4 parts of water), a significant reduction in pathogen levels may be reached with an average contact time of 5 minutes (Abaidoo, R. C. *et al*, 2010). However, this seems to be an expensive and impractical way in house kitchens, therefore, in my experiment, at lower concentrations of vinegar, the acetic acid concentration was changed to assess its impact on reducing coliforms and specifically *E.coli*.

Finally, I can state that my hypothesis is true as acetic acid reduce the coliform bacteria in the food in traditional cleaning practices although it would not completely eliminate the risks of foodborne diseases with its limited impact on microorganisms at the taken concentrations.

I can refer to a new research question after completing the experiments as there are a number of liquid sanitizers to be used to decrease bacterial load: How do different concentrations of *Sodium hypochlorite* impact the presence of *E.coli* in *L.sativa*, and *P.crispum* samples during cleaning process? *Sodium hypochlorite* (NaOCl), also known as food grade bleach, is available as liquid sanitizer that is mixed with water and used immediately to control microbial growth. NaOCl requires minimal monitoring, and is a common chlorine source in small scale operations like sanitizing fruits, vegetables and fresh-cut produce (Gehringer, R. A. *et al*, 2017).

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APPENDIX I: Agar specification used in the trials

VRB (Violet Red Bile Lactose) agar

acc. ISO 4832 and FDA-BAM GranuCult®

VRB (Violet Red Bile Lactose) agar MSDS (material safety data sheet) or SDS, CoA and CoQ, dossiers, brochures and other available documents.

- SDS
- Brochures
- Certificates
- CoA
- Data Sheet

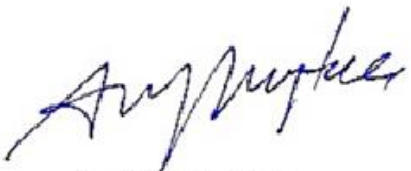
Synonyms: Crystal-violet neutral red bile agar, VRB-agar (Crystal-violet neutral-red bile agar)

ph Value: 7.3 - 7.5 (40 g/l, H_2O, 37 °C) (after autoclaving) **Solubility:** 39.5 g/l

Source: https://www.merckmillipore.com/BE/en/product/VRB-Violet-Red-Bile-Lactose-agar,MDA_CHEM-101406. Accessed on 30 September 2021.

APPENDIX II: Signed letter by the supervisor

has done her IB diploma programme extended essay experiment in microbiology laboratory of the Hacettepe University Food Engineering Department in person under my supervision.



Prof. Dr. Aykut Aytaç

Hacettepe University

Food Engineering Department

APPENDIX III: Pictures from the experiment

Figure-1: Washing vegetable samples in different vinegar solutions

Figure-2: Preparation of vegetable samples in stomacher bags

Figure-3: Counting colonies in VRB agar

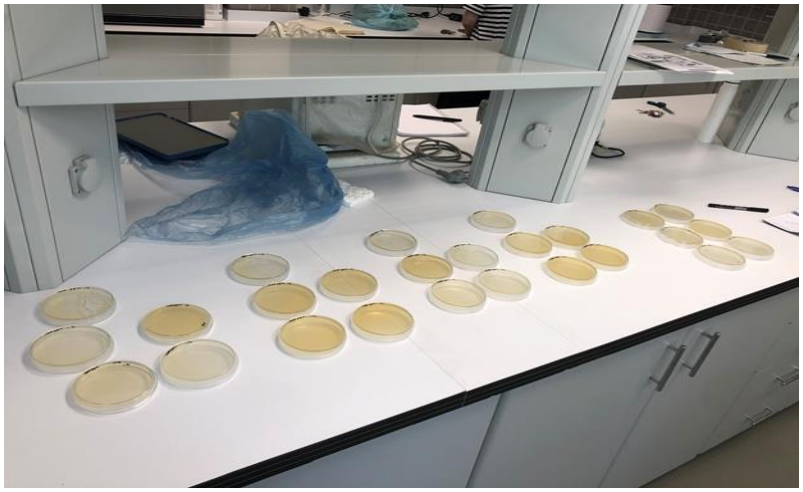


Figure-4: Petri dishes to be counted after incubation