

Introduction

E. coli K12 is found naturally in the intestines of humans and animals, however tea leaves are vehicles of transmission due to improper production handling and field contamination (Taniuchi et al. 2012). *E. coli* K12 can grow at temperatures ranging from 4°C to 45°C. Cold brewing from black tea leaves is performed at or below 15°C, allowing for the survival and growth of any *E. coli* K12 (Balentine et al. 2002). Tea originates from the plant *Camellia sinensis*, and grows in acidic soil conditions. *E. coli* K12 is resistant to acidic conditions, and is able to adhere to the acidic surface of black tea leaves (Schwalfenberg et al. 2013). Many producers are finding that products they originally thought were at low risk for *E. coli* contamination are indicating minimal to high amounts of pathogenic growth. Little is known about the presence of *E. coli* in pre-dried products, however this becomes increasingly important in developing countries with little food security.

Objective

- The objective of this study was to investigate the reduction of *E. coli* through thermal processing for 1 and 5 minutes at 73.9°C on dried black tea leaves.

Materials & Methods

- Two treatments, along with positive and negative controls were used in this 10-day confirmation study.
- Both treatments investigated used thermal processing via an incubator. Treatment 1 consisted of bringing the product to 73.9°C and held for 1 minute. Treatment 2 was brought to 73.9°C and held for 5 minutes.
- The negative control was not inoculated with *E. coli* K12. The positive control, Treatment 1, and Treatment 2 were all inoculated with *E. coli* K12.
- The 10 gram samples were treated, stomached in 90ml of peptone, and diluted to prepare for plating.
- A total of 270 media plates each with either McConkey agar for *E. coli* growth, PDA agar for yeast and mold growth, or PCA agar for total colony formation.
- On Day 0, 18 McConkey plates, 18 PDA plates, and 18 PCA plates were inoculated with positive and negative controls.
- On Day 2 and Day 7, 36 McConkey plates, 36 PDA plates, and 36 PCA plates were inoculated with either a positive control, negative control, Treatment 1 sample, or Treatment 2
- On Day 10, after all media was incubated at temperatures optimal for microbial growth, data was taken by counting colony growth on each plate.



Figure 2: Black Tea Leaves used in this study.

Results

Table 1: Summary of Data

Item	Treatment				p-value
	Negative Control	Positive Control	One minute Treatment	Five minute Treatment	
<i>E. Coli</i>	n/d	5.2	4.0	2.6	0.047
Total Colony	2.4	2.9	2.5	2.5	0.006
Yeast & Mold	n/d	2.7	1.9	n/d	0.032

- Out of the 36 *E. coli* plates plated for Treatment 1 and 2, only 2 had countable growth (5.5%). Though it was countable, the growth was minimal at only 30 CFU's/ml.
- The combined treatment's p values were ≤ 0.05 indicating strong evidence against the null hypothesis.
- By performing a confirmation study, we were able to prove that heat treatment is an effective way to eradicate *E. coli* on tea leaves.
- In this confirmation study, the heat treatment used was successful in reducing *E. coli* K12 by a 3-log reduction.

- As can be seen below there was a slight difference in the *E. coli* reduction in Trial A and Trial B.
- The slight increase can be attributed to a longer *E. coli* incubation time between plating, and therefore a higher *E. coli* count before the kill step.

Growth of *E. coli* by Trial

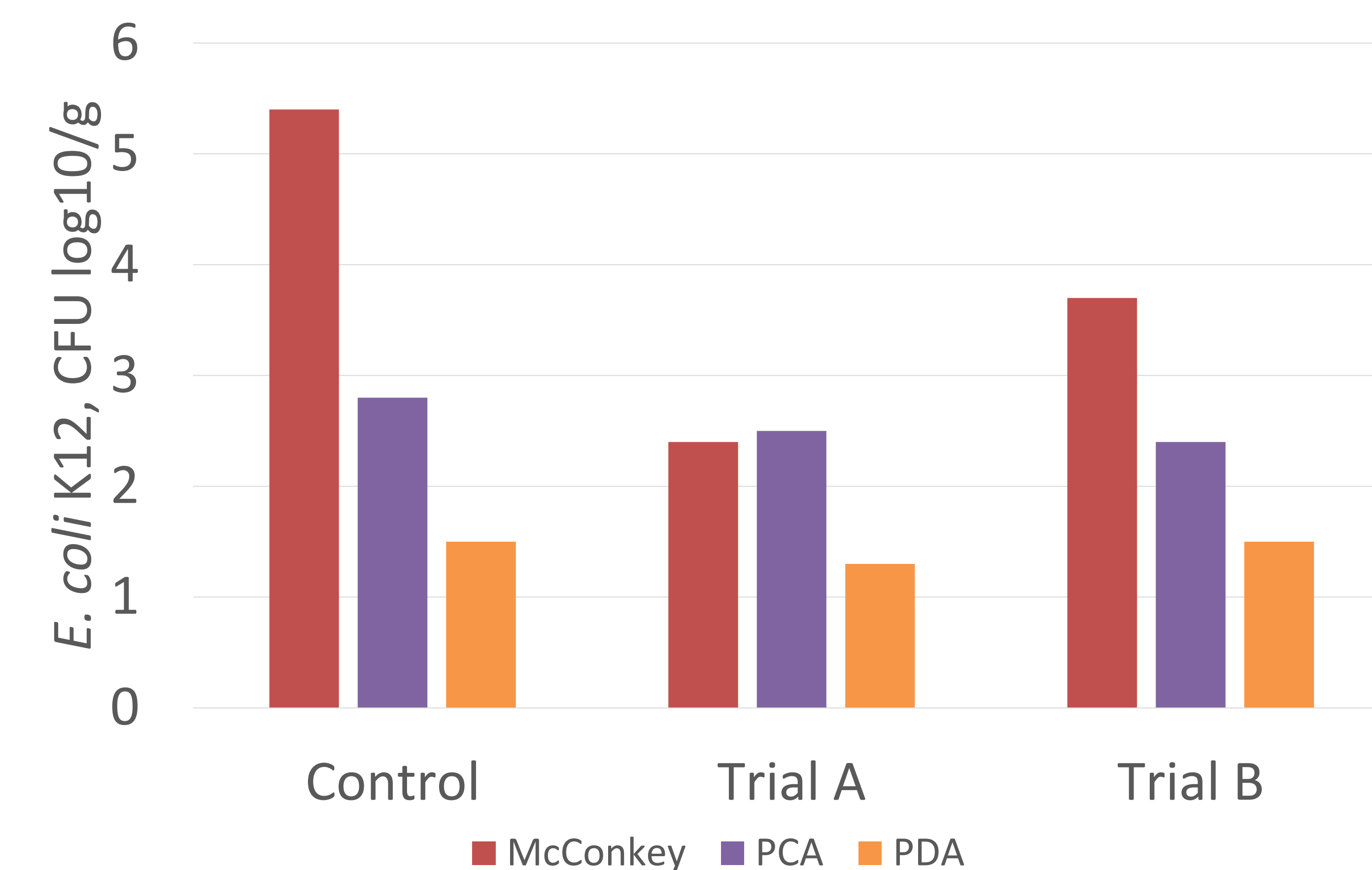


Figure 1: Growth of *E. coli* by Trial

Conclusions

- From this study, it can be concluded that thermal processing of dry black tea can prevent *E. coli* from surviving in final products whether consumers decide to brew their tea via cold brew or hot brew. This will prevent the consumers from having to perform the kill step in order to eliminate *E. coli* in the final product. Both of the thermal processing treatments were effective at killing the *E. coli* K12 in the tea leaves. The longer the heat treatment time, the more *E. coli* K12 bacteria was able to be killed along with yeast and molds.

References

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- Taniuchi, M., Walters, C. C., Gratz, J., Maro, A., Kumburu, H., Serichantalergs, O., ... Houpt, E.R. (2012). Development of a multiplex polymerase chain reaction assay for diarrheagenic *Escherichia coli* and *Shigella* spp. and its evaluation on colonies, culture broths, and stool. *Diagnostic Microbiology and Infectious Disease*, 73(2), 121-128. doi:10.1016/j.diagmicrobio.2012.03.008