



Effects of the different time length of heat treatments in *E. coli* K12 in raw shelled walnuts



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Introduction

Escherichia coli, (*E. coli*), are found in the environment, in animals, and are on everyday food item. *E. coli* is diverse and can live in different environments, “Person-to-person contact, environmental exposure and drinking or swimming in contaminated water are also thought to be important sources of infection” (6), which is why it is such a danger to the world. *E. coli* is linked to high water activity foods, such as meat, the “consumption of . . . ground beef has been linked epidemiologically with several outbreaks of disease caused by *E. coli*” (3). For this experiment, walnuts, a low water activity (Aw) food, was picked because of the recent outbreak in 2011, “13 patients from three provinces have been sickened so far in an *E. coli* O157:H7 outbreak linked to potentially tainted walnuts” (1). Walnuts, are used in baked goods, and *E. coli* is “destroyed by pasteurization” (7). In another experiment “many examples of heated *E. coli* O157:H7 cells showing sublethal damage” (6). With this knowledge, and with the 2011 outbreak, walnuts and thermal heat processing were chosen to reduce the growth of *E. coli*, and to help reduce the growth of yeast and molds.

Objective

The objective of the study was to evaluate if the roasting treatment with 10 and 20 minutes at 140°C in raw shelled walnuts is able to reduce the number of *E. coli* with 3 log reduction.

Materials and Methods

This study involved four treatments: negative control, positive control, and two interventions. The negative control was non-inoculated walnuts and the positive was walnuts inoculated with 10⁷ CFU/ml of *E. coli* K12. The interventions were roasting the walnuts at 140° C for 10 minutes and 20 minutes. The control and intervention samples were spread plated on three types of media; PDA (Yeasts and Molds), PCA (Total Plate Count), and McConkey agar (*E. coli*), with three replications each, and incubated for 48 hours at 35° C. This study was conducted over one week with the controls plated on day 0 at 10⁻¹, 10⁻², 10⁻³ dilutions. All four treatments were plated on day 2 and 7 at 10⁻², 10⁻³, 10⁻⁴ dilutions. The colony counts were recorded and analyzed using SAS GLIMMIX v9.4.

Reference's

(1)05, 2011 Apr. “NEWS SCAN: Walnut E Coli Infections, H1N1 Vaccine in Preterm Kids.”CIDRAP, University of Minnesota, 5 Apr. 2011, www.cidrap.umn.edu/news-perspective/2011/04/news-scan-walnut-e-coli-infections-h1n1-vaccine-preterm-kids. (2)Clermont, O., et al. “Rapid and Simple Determination of the Escherichia Coli Phylogenetic Group.” Applied and Environmental Microbiology, vol. 66, no. 10, Jan. 2000, pp. 4555–4558., doi:10.1128/aem.66.10.4555-4558.2000. (3)Fratamico, P.m., and A.g. Gehring. “ESCHERICHIA COLI O157 | Escherichia Coli O157 and Other Shiga Toxin-Producing E. coli.” Encyclopedia of Food Microbiology, 2014, pp. 740–747., doi:10.1016/b978-0-12-384730-0.00104-x. (4)Nelson Wyatt. (2011, Apr 7,). One quebecer dead, 13 other people ill after being stricken by E. coli. The Canadian Press (5)Pelczar, M. (2016). Summary. Analysis, 76(4), 449-453. doi:10.1093/analys/anw034 (6) Stringer, S.c., etal. “Thermal Inactivation of Escherichia coliO157:H7.” Journal of Applied Microbiology, vol. 88, no. S1, 2000, doi:10.1111/j.1365-2672.2000.tb05335.x. (7)Trinetta, V. (8)Yang, J., Pan, Z., Takeoka, G., Mackey, B., Bingol, G., Brandl, M. T., . . . Wang, H. (2013). Shelf-life of infrared dry-roasted almonds. Food Chemistry, 138(1), 671-678. doi:10.1016/j.foodchem.2012.09.142

Results

Table 1-Effects of Roasting on Microorganisms Growth Over Time

	Media	Days	Negative Control	Positive Control	10 Minutes	20 Minutes	p-value
CFU log10/g	PCA	0	n/d ^c	5.2 ^a	-	-	<0.0001
		2	n/d ^c	4.9 ^a	n/d ^c	n/d ^c	
		7	n/d ^c	3.3 ^b	n/d ^c	n/d ^c	

Table 2-Effects of Roasting on *E. coli*

	Media	Negative Control	Positive Control	10 Minutes	20 Minutes	p-value
CFU log10/g	McConkey	n/d ^b	3.4 ^a	n/d ^b	n/d ^b	0.0002
	PDA	2.4 ^b	3.9 ^a	2.5 ^b	2.5 ^b	

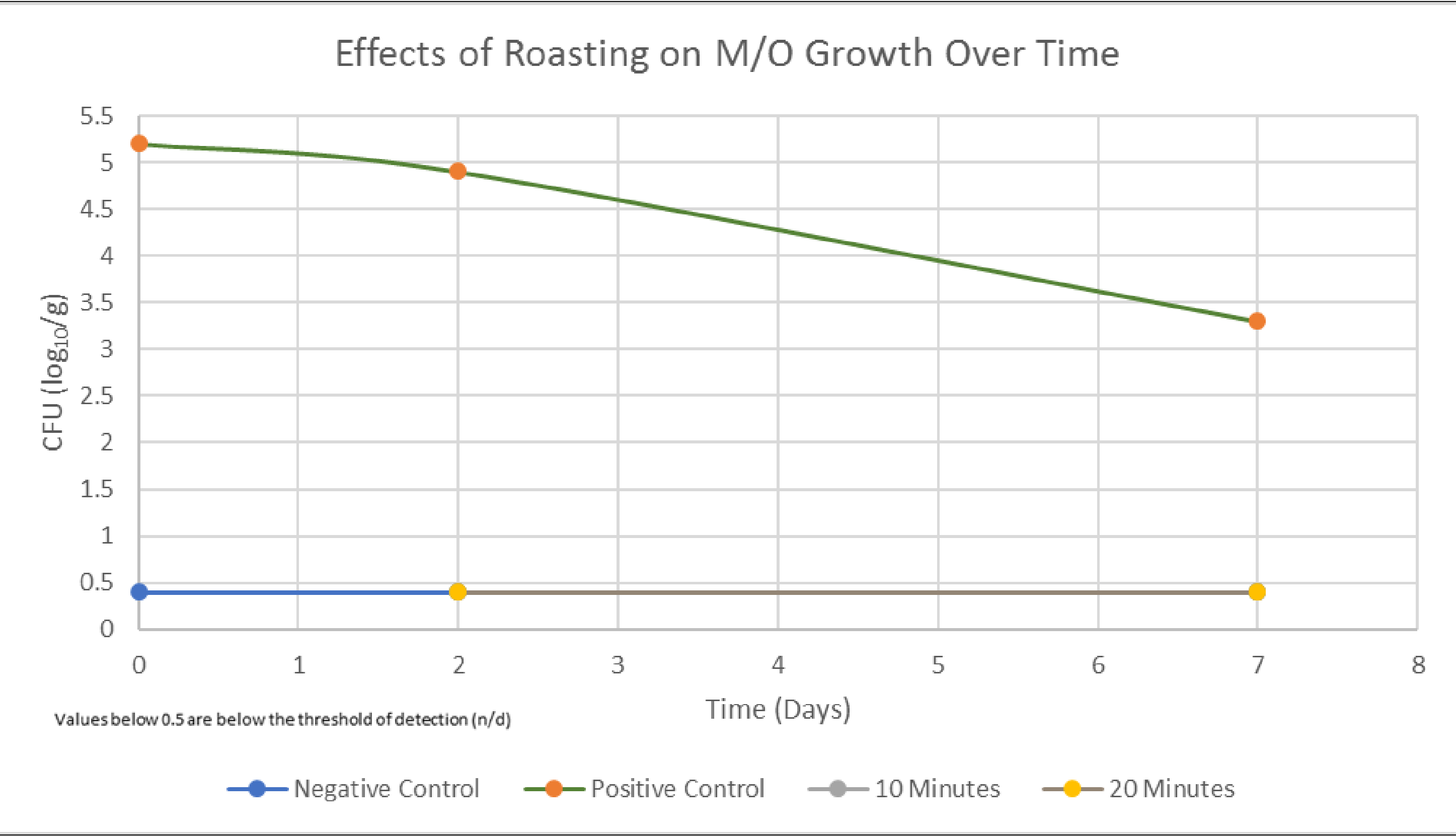


Figure 1-Effects of Roasting on Microorganisms Growth Over Time

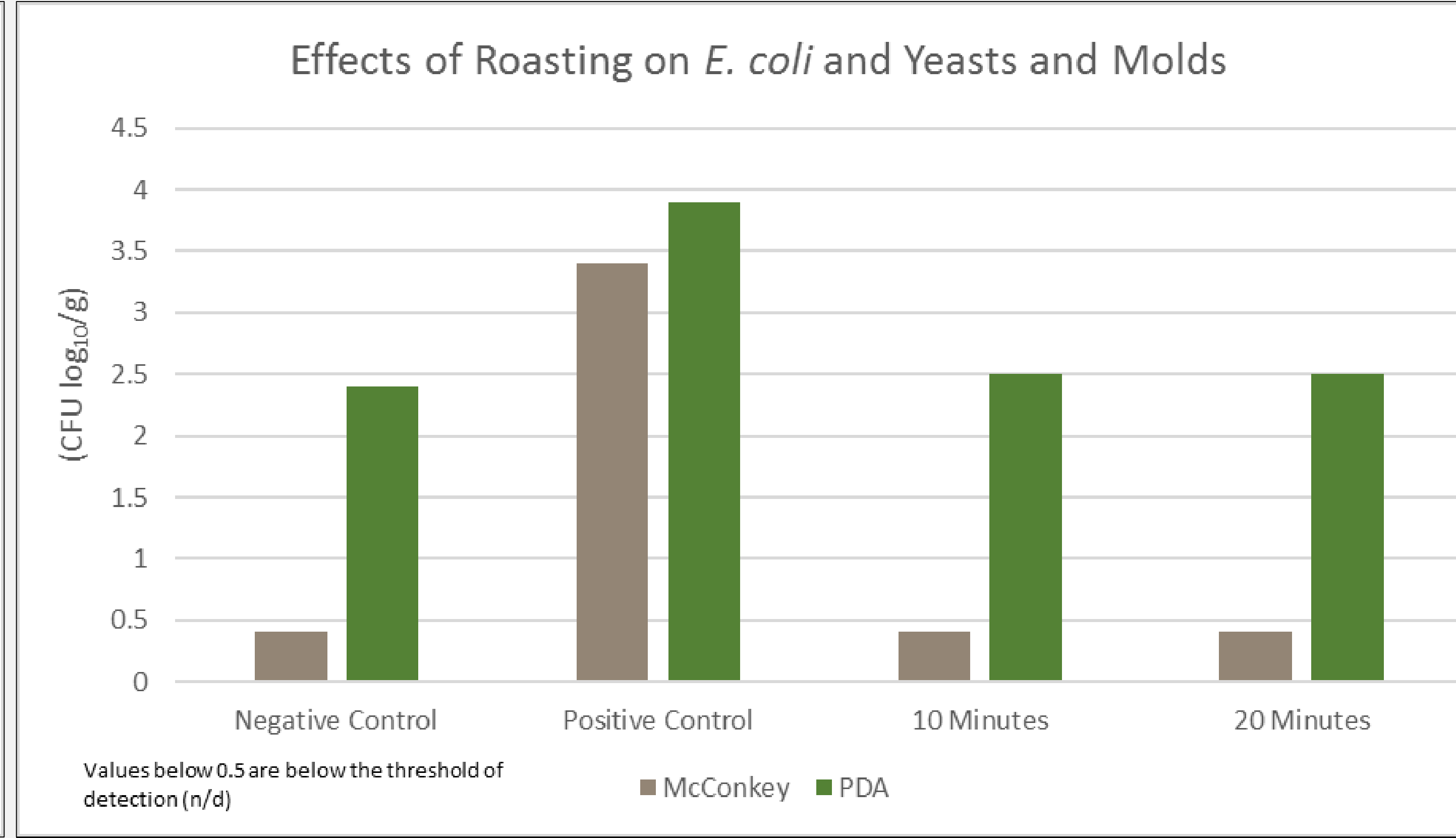


Figure 2- Effects of Roasting on *E. coli* and Yeasts and Molds

- * With PCA plates there is a reduction in the CFU count with the treatments of 10 and 20 minutes of roasting with a P-Value of <0.0001. But there is no difference in-between each treatment. As stated in Table 1, many of the values are not detectable (n/d) meaning they were below level of detection of 0.5 CFU log10/g.
- * But there was a similar value in the superscript of the n/d of the negative control and that of each treatment, as shown in Chart 2. Showing that the treatments could achieve the same “level” of microorganism's (M/O) on the walnuts as what was present in the negative control.
- * Table 2 shows the effect of each treatment on the growth of either *E. coli* and/or Yeast and Mold on walnuts. On the PDA plates, which are representative of yeast and molds, one can observe a 1.4 log reduction from positive control to 10-minute and 20-minute treatments.
- * Both treatments were effective in lowering the growth of M/O as indicated by the P-Values in Table 2.
- * As previously stated, many of the data points were indicated as below the detectable level of 0.5 CFU log10/g the shared similar superscript values indicate a similarity. Once more, Chart 1 shows that the n/d values are like that of the negative control and therefor show a maximum log reduction of 1.4.

Conclusion

The results of this study show that both roasting with 10 minutes and 20 minutes at 140°C significantly reduce *E. coli* in raw shelled walnuts. There was contamination in some PDA negative controls which not consistent to total plate count negative controls. The contamination could occur from yeast and mold in the environment. Laboratory practice re-training is suggested. In conclusion, we reject the null hypothesis that roasting with 10 minutes and 20 minutes at 140°C is not able to reduce *E. coli* proliferation in raw shelled walnuts.