**Inactivation of *Toxoplasma gondii* on Blueberries using Low Dose Irradiation without
Affecting Quality**

Alison Lacombe¹, Anna Breard², Cheng-An Hwang³, Dolores Hill⁴, Xuetong Fan³, Lihan
Huang³, Byong Kwon Yoo³, Brendan A. Niemira¹, Joshua B. Gurtler¹, and Vivian C.H. Wu^{5*}

¹Food Safety and Intervention Technologies Research Unit, Eastern Regional Research Center,
United States Department of Agriculture. 600 Mermaid Lane, Wyndmoor, PA 19038.

²School of Food and Agriculture, University of Maine. 5735 Hitchner Hall, Orono, ME 04469.

³Residue Chemistry and Predicative Microbiology Research Unit, Eastern Regional Research
Center, United States Department of Agriculture. 600 Mermaid Lane, Wyndmoor, PA 19038.

⁴Animal Parasitic Diseases Laboratory, Beltsville Area Research Center, United States
Department of Agriculture. 10300 Baltimore Avenue, Beltsville, MD 20705.

^{5*} Produce Safety and Microbiology Research Unit, Western Regional Research Center,
Agricultural Research Service, United States Department of Agriculture. 800 Buchanan Street,
Albany, CA 94710.

* Author for corresponding:

Vivian C.H. Wu, Ph.D.

Tel.: 510-559-5829; Fax: 510-559-6165

E-mail address: vivian.wu@ars.usda.gov

Abstract:

Toxoplasma gondii is a common protozoan parasite, whose environmentally-resistant stage, the oocyst, can contaminate irrigation water and fresh edible produce. Current washing steps in produce processing may not be effective for eliminating *T. gondii* from at-risk varieties of produce. The objective of this study was to evaluate low-dose irradiation as a means to inactivate *T. gondii* oocysts on blueberries. Blueberries (10 g) inoculated with *T. gondii* (5 log oocysts/g) were exposed to an absorbed dose of 0 (control), 0.2, 0.4 or 0.6 kGy gamma radiation at 4°C. After treatment, oocysts were recovered from berries by washing, and excysted sporozoites were enumerated using a plaque assay. Vero cells were maintained as monolayers in a 6-well microplate, exposed to sporozoites recovered from berries, and incubated for 7 days to determine plaque-forming units (PFU). Immediately after treatment, blueberries without inoculated *T. gondii* were analyzed for compression firmness, surface color, and total anthocyanins. The results from this experiment demonstrate that the viability of *T. gondii* oocysts was significantly reduced after an irradiation treatment of 0.2 kGy. All treatments produced reductions of 4 log PFU/g beyond the detection limit of 1 log PFU/g. Quality analysis showed that there was no significant change in compression firmness, anthocyanins, or color in berries after the irradiation treatment. Results of this study indicated that low-dose irradiation is a potential intervention measure for controlling *T. gondii* contamination on blueberries without affecting product quality.

Keywords: *Toxoplasma gondii*, parasite, blueberry, irradiation, food safety and quality

1. Introduction:

The U.S. Food and Drug Administration (FDA) has recently identified fresh fruits and vegetables as commodities with a high potential risk of *Toxoplasma gondii* contamination (Cole et al., 2011; FDA, 2012). These commodities are frequently consumed fresh, ready-to-eat, or used as ingredients, and are generally minimally processed to maintain freshness, nutritional value, and sensory quality. Increasing consumption of produce has resulted in more outbreaks of foodborne illnesses linked to parasites (CDC 2011). The Center for Disease Control and Prevention has recognized *Toxoplasma gondii* as an emerging food safety threat due to the increasing complexity of the food supply (CDC, 2011). It is estimated that nearly one-third of humanity has been exposed to *T. gondii* (Dubey, 1996). In the United States, *T. gondii* is one of the leading causes of hospitalization and foodborne illness-related death, ranked just below *Salmonella*, norovirus and *Campylobacter* in cases of infection (Batz et al., 2012; CDC, 2011). Traditionally, foodborne *T. gondii* infection has been associated with feline contamination and undercooked meat; however, there have been cases of toxoplasmosis linked to produce (Ekman et al., 2012).

Parasites are one of the most common etiological agents that contaminate berries (Sivapalasingam et al., 2004). Raspberries and strawberries have been implicated in several multistate outbreaks of cyclosporiasis. The berries were likely contaminated on the farm by inadequately-treated water (Sivapalasingam et al., 2004). Seasonal cyclosporiasis among family members of field workers in Guatemala has been documented, potentially contaminating the fruit directly with parasites during harvesting or packing (Sivapalasingam et al., 2004). In addition, *T. gondii* oocysts found outside of the human host, such as in water and soil, are more infectious

67 than those found in freshly excreted feces (Kniel et al., 2002). Currently, there are no specific
68 guidelines for the food industry to follow in regards to controlling *T. gondii*. Guidelines for
69 preventing parasite contamination in food are mainly focused on other microorganisms, such as
70 *Trichinella spiralis* in pork (Dubey et al., 1990). Given the limited research that has been
71 conducted on interventions for *T. gondii*, the USDA recognizes that more research is needed for
72 this emerging food safety pathogen. Governments, international aid agencies, and institutions are
73 taking initiatives for the future development of small-scale agriculture with regards to the
74 management of resources and irrigation practices (Yannopoulos et al., 2015). Irradiation has
75 been previously evaluated for control of *T. gondii* tissue cysts in meat, demonstrating its
76 potential as an effective intervention (Dubey, 1996; Dubey, et al., 1998; Dubey and Thayer,
77 1994; Ware et al., 2010). The U.S. federal regulations have set radiation limitations for several
78 food products. A dose not exceeding 4 kGy is allowed for control of foodborne pathogens and
79 shelf-life extension in fresh, iceberg lettuce, and spinach (FDA, 2012). Prevention methods for
80 *T. gondii* oocyst contamination are needed for the safety of fresh produce; however, there is a
81 lack of comprehensive research for fruit and vegetables using irradiation as a control for surface
82 contaminating *T. gondii* oocysts. Currently, there has been no comprehensive evaluation of the
83 effect of irradiation of *T. gondii* on the surface of blueberries or an investigation into its effect on
84 food quality. Identifying a range of irradiation doses which are within the allowable limits for
85 berries that are effective against surface contaminating *T. gondii* oocysts and do not impact food
86 quality would be useful in streamlining food safety regulations (FDA, 2012). More studies are
87 needed on using irradiation for control of *T. gondii* in fruits and vegetables to establish doses that
88 are effective against *T. gondii*, within the permitted dose ranges, and not detrimental to product
89 quality.

Toxoplasma gondii was recently ranked very highly in burden of disease estimates for foodborne pathogens in the Netherlands and the USA (Havelaar et al., 2012; Hoffmann, et al., 2012). Until recently the only interventions for parasites on fresh produce have been preharvest management of resources, such as water treatment and increased worker hygiene (El Said Said, 2012). Blueberries provide a good source of anthocyanins in the American diet, therefore the control measurements should not adversely affect this functional nutrient (Wu et al., 2006). Heat treatments are not appropriate for blueberries because of their heat liable nutrients, therefore non-thermal interventions are urgently needed. Irradiation offers a nonthermal intervention against foodborne pathogens, has a low contact time, and does not require chemical inputs (Dubey, 1996). Accordingly, the objectives of this study were to: 1) investigate the efficacy of low-dose irradiation against *T. gondii* oocyst contamination on the surface of blueberries, and 2) determine the effect of low-dose irradiation on treated berries' compression firmness, surface color, and total anthocyanins in blueberries.

2. Material and Methods

2.1 Preparation of *T. gondii* oocysts

T. gondii oocysts (ME49 strain) were obtained from the USDA Animal Parasitic Diseases Laboratory, Beltsville, Maryland. Oocysts were isolated from the feces of infected cats beginning at three days post-infection with orally inoculated tissue cysts from mouse brains (Dubey et al., 2009). A fecal water slurry (1:5) was prepared, filtered through cheesecloth (Fisher Scientific, Pittsburgh, PA, USA), and then filtered through a series of 250, 150, 75, and 45-micron sieves (Fisher Scientific) to remove debris. The slurry was washed with water several times by centrifuging at 1,000 rpm for 15 minutes, followed by removing the supernatant, and re-

suspending the pellet in water. The final pellet was re-suspended in 2 M sucrose solution (Fisher Scientific) and centrifuged at 2,000 rpm for 10 minutes. The top 6 ml of the resulting solution were drawn off and pooled. Aliquots of 50 ml of pooled material were washed with water and centrifuged at 2,000 rpm for 10 minutes to remove sucrose. The resulting supernatant was removed (about 45 ml in volume) and centrifuged at 2,000 rpm for 10 minutes. The pellet was re-suspended in water with 2% sulfuric acid (Fisher Scientific) was added as a preservative. The oocyst preparation was gently shaken at room temperature for 1 week until sporulation was complete and stored at 4°C until used.

2.2 Inoculation of Fresh Blueberries

Blueberries were obtained from local grocery stores on the day of experiments and were discarded after 48 h. Samples were inspected for deformities and firmness, wiped cleaned with a Kimwipe (Kimberly Clark, Roswell, GA), and dipped in 70% ethanol (Fisher Scientific) to eliminate background microbiota. Blueberries (10 g) were dip-inoculated with 500 µl of 10⁵ PFU/g of *T. gondii* oocysts, vortexed for 1 min, and stored at 4°C for 12-18 h.

2.3 Irradiation of *T. gondii* oocysts on the surface of blueberries

A self-contained Lockheed Corporation ¹³⁷CS gamma radiation source at a dosing rate of 0.075 kGy/min was utilized. Blueberries inoculated with oocysts were exposed to absorbed doses of 0.0, 0.2, 0.4 and 0.6 kGy at 4°C. The dose rate of 0.075 kGy/min was confirmed with NIST calibration using alanine pellet dosimeters. Times for treatments were calculated according to these dosage rates. Alanine pellet dosimeters were used with each irradiated sample.

2.4 Blueberry processing for obtaining excysted *T. gondii* oocysts after irradiation

After irradiation treatments, samples were processed to obtain excysted *T. gondii* oocysts following protocols of (Kniel et al., 2002; Lindsay et al., 2008; Ware et al., 2010). First, 10 ml

of Dulbecco's phosphate-buffered saline (DPBS; Fisher Scientific) was added to each sample and hand massaged/shaken for 60 s in a filtered stomacher bag (Nasco, Fort Atkinson, WI, USA). The filtrate was collected and placed in a sterile 15 ml centrifuge tube (Fisher Scientific) and centrifuged at 13,500 x *g* for 10 min. The supernatant was removed and the pellet was resuspended in 1 ml of DPBS. The suspension was placed into a sterile 2 ml tube (Fisher Scientific) containing 0.5 g of 0.5 mm glass beads (Biospec Products, Bartlesville, OK, USA) and mechanically disrupted with a bead beater (Mini Beadbeater, Biospec Products) for 60 s. Then 500 µl of pre-warmed freshly prepared 10% bovine bile (Fisher Scientific) was added to the suspension and incubated in a 37°C water bath for 1.5 h. The solution was removed and placed into a new sterile 15 ml centrifuge tube and centrifuged at 13,500 x *g* for 10 minutes. The supernatant was removed and the pellet re-suspended in 1 ml Dulbecco's Modified Eagle Medium (Fisher Scientific).

2.5 Determination of viability of *T. gondii* oocysts and tissue cysts

Viability of irradiated *T. gondii* sporozoites (the parasite stage contained within the oocyst) was evaluated using cell culture-based plaque assay (Saadatnia et al., 2010). Vero cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in Minimum Essential Media supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine (Life Technologies, Carlsbad, CA, USA) at 37°C with 5% CO₂. Vero cells were maintained as monolayers in 150-cm² or 225-cm² flasks (Corning Inc., Tewksbury, MA, USA) and seeded at 5 x 10⁵ cells onto 6-well plates (BD Falcon, Franklin Lakes, New Jersey) at 2 ml/well. Cell monolayers exposed to dilutions of excysted *T. gondii* sporozoites, and were incubated undisturbed at 37°C and 5% CO₂ for 7 days before plaque enumeration.

2.6 Evaluation of blueberry quality after irradiation

Overall appearance of blueberries after irradiation treatments were evaluated and the total anthocyanins in treated berries were measured using a pH differential method (Lee et al., 2005). Potassium chloride (Fisher Scientific) adjusted to pH 1.0 and sodium acetate (Fisher Scientific) adjusted to pH 4.5 were prepared. Each solution at 270 μ l was added to berries (30 μ l) that were homogenized in 25 ml of methanol and filtered (Lee et al., 2005). The samples in a 96-well microplate were read at 525 nm and 700 nm using a spectrophotometer (Biotech, Winooski, VT, USA). The difference in absorbance was used as indication of relative anthocyanin contents, based on structural changes of the anthocyanin chromophore between pH 1.0 and 4.5. The contents were calculated according to Lee et al. (2008) and reported as equivalents of cyanidin 3-galactoside (C3G). Surface color of blueberries was measured on opposite sides of each berry by a Ultra Scan VIS color analyzer (Hunter Labs, Reston, VA, USA) using Hunter scale (L^* , a^* , and b^*) calibrated with a white tile. For firmness, each berry was placed on its side and compressed at a constant speed of 0.5 mm/s that measured the mm of deflection between the minimal (0.15 N) and maximal (1.96 N) force thresholds using a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY, USA). Firmness was reported as the mean force (N) to deform the surface of the fruit by 1 mm.

2.7 Statistical Analysis

Reductions of *T. gondii* oocyst by irradiation were calculated based on three repeats (2 replicates, $n = 6$) and expressed as the differences between the number of PFU of treated samples and non-treated samples. Five repeats of each experiment were performed for compression firmness (5 replicates, $n = 25$), surface color (10 replicates, $n = 50$), and total anthocyanins (three replicates, $n = 15$). Analysis of variance (ANOVA) was performed to determine the effect of

irradiation dose on oocyst reduction and berry quality using the JMP 9 platform (SAS Institute, Cary, NC). Tukey's Honest Squared Difference was used to examine the differences among treatments means. Significant differences were defined at $p < 0.05$.

3. Results and Discussion

3.1 Reduction of *T. gondii* on blueberries by irradiation

This is the first study to investigate the use of irradiation to inactivate sporulated *Toxoplasma* oocysts on the surface of blueberries. The results from this experiment demonstrate that viability of *T. gondii* oocysts was significantly reduced after an irradiation treatment of 0.2 kGy (Figure 1). All irradiation treatments produced reductions of 4 log PFU/g (detection limit was 1 log PFU/g). Previous research demonstrated that for unsporulated oocysts in solution, a Gamma irradiation dose as low as 0.4 kGy was effective at eliminating viability, while other studies demonstrated that sporulated oocysts in solution could be controlled by even lower doses of 0.25 kGy (Dubey et al., 1998).

Previous research into human infection with zoonotic protozoal parasites focused on *Toxoplasma* transmission by the consumption of meat products. The contamination of fresh produce by parasites was thought to be limited and confined to distinct geographic regions because of the adaptations of parasites to specific hosts, select intermediate hosts, and particular environmental conditions (El Said Said, 2012). However, with the growing complexity of the food supply, the barriers that previously prevent the dispersal of parasites to new geographic regions and to new susceptible hosts are slowly being breeched. The increased risk of contamination has been facilitated by the expansion of international trade and the rapid increase in trades of refrigerated foods (El Said Said, 2012). In addition, consumers in the U.S. have

shifted towards the consumption of fresh food (i.e., raw or minimally processed), which bypasses important preparatory measures that inactivates parasites.

Irradiation has proven to be a successful intervention against *T. gondii* contamination for tissue cysts in meat (Dubey et al., 1998) and, in this study, oocysts on blueberries. Irradiation's high energy breaks chemical bonds in molecules that are needed for microbial growth and integrity, therefore microbes die or can no longer replicate (Dubey et al., 1990). In contrast, *T. gondii* oocysts are resistant to destruction by many chemical sanitizers, the currently used non-thermal interventions for berries. As part of post-harvest processing, vegetables and fruits (including blueberries) are commonly washed or sprayed with chlorinated water containing 50–200 ppm of active chlorine in order to reduce microbial loads (Wu and Kim, 2007). Recent studies demonstrated that chlorine at high concentrations may not be effective in reducing *T. gondii* oocyst contamination on fresh produce (Wainwright et al., 2007). In addition, there was no significant difference in survival between untreated oocysts and those treated with iodophor-based detergent, acidified ethanol, sodium hypochlorite, or ozone (Villegas et al., 2010; Wainwright et al., 2007).

3.2 Blueberry quality after irradiation

The effect of irradiation dosage at 0.2-0.6 kGy on blueberry quality has not been evaluated. This study was the first one to investigate the effect of irradiation on anthocyanins in blueberries. These results suggest that low-dose irradiation can be effectively applied on blueberries without the concern of degradation of product quality. Anthocyanins reside in vacuoles mostly found on the skin of blueberries and are prone to denaturation by heat, light, and oxygen radicals (Bjoroy et al., 2009). The stability of anthocyanins in blueberries is influenced by a number of factors. The temperature of treatment influences the stability of anthocyanins by

affecting the enzymatic activity of polyphenoloxidase (PPO) and β -glucosidase, which accelerates the rate of degradation of anthocyanins (Patras et al., 2010). Anthocyanins are prone to thermal degradation with significant reduction of C3G occurring around 38 °C (Patras et al., 2010). Results of total anthocyanin and texture analyses (Figure 2) show that there were no significant differences between irradiated samples and the control. Trend analysis suggests that anthocyanins may be reduced at higher doses of irradiation ($R^2 = 0.859$) while compression demonstrated no trend toward softening ($R^2 = 0.098$). The high energy of Gamma irradiation can break chemical bonds in food, causing chemical changes in food quality (EPA, 2014). The degradation mechanism for anthocyanin suggests that oxidation plays an important role in the oxidative cleavage of chromophores, through breakdown of conjugated double bonds (Tiwari et al., 2009). Mitigation of anthocyanin loss through enzymatic degradation, thermal deterioration, and other chemical reactions can be achieved through optimization.

Blueberry color is a complex quality characteristic affected by anthocyanin content, quantity and structure of surface waxes (Saftner et al., 2008). Surface color measurements were not significantly impacted for the L^* and a^* values and b^* values (Table 1). Decreases in the L^* values reflect a darkening of the blueberry surface color, which could be a result of the melting of the blueberries' cutaneous wax. The increases in a^* and b^* values represent an increase of the perceived surface red and blue colors, respectively. Recent studies have attempted to correlate surface color with overall consumer acceptability (Saftner et al., 2008). Surface L^* values were negatively correlated with sensory scores for intensity of blue color and weakly correlated with sensory scores for acceptability of appearance. The present study suggests that irradiation does not affect color, which is an important factor in consumer acceptability.

3.3 Irradiation as a viable intervention strategy against *T. gondii*

The results of this study indicate that low-dosage irradiation is effective for decontaminating *T. gondii* oocyst on blueberries. The doses are well within the current allowable irradiation dose and are in line with current hurdle practices that utilize lower levels of multiple treatments to control foodborne pathogens and maintain product quality (FDA 2012). Food irradiation is a physical method of processing food and has the potential for decontaminating fruits and vegetables to meet quarantine requirements for export trade (FDA 2012).

The United States imports large quantities of blueberries in the winter months. Southern hemisphere exporters, such as Chile, are major sources of these imports. The United States Department of Agriculture-Animal and Plant Health Inspection Service has imposed restrictions and mandatory fumigation requirements for imported berries to prevent the introduction of invasive species that may accompany the imported berries (EPA 2014). Regulatory discrepancies among the countries that have approved irradiated foods hampers the introduction of irradiation treatment for international trade. Low doses of irradiation can reduce spoilage losses of food products and enhance the food safety by eliminating pathogenic microorganisms in food products. In addition, irradiation may successfully replace fumigation treatments. One of the most important advantages of irradiation processing is that it is a non-thermal treatment that does not significantly alter physicochemical characteristics of treated product while penetrating the entire product. It can be applied to food after packaging, so further handling contamination is avoided. It should be noted that irradiation is a capital-intensive process, therefore adequate product volume must be made available to maximize the use of the facility and minimize the unit cost of treatment.

There is a need to increase the awareness of the impact of parasites on food safety. This includes recognizing parasites' potential effect on public health, health care as well as economic consequences such as worker productivity and agricultural losses. With the growing complexity of the food supply, non-thermal treatments that do not impart adverse effects on food quality, like irradiation, will become more important in controlling foodborne parasites on fresh produce. Future research should include pilot-sized studies to validate the low doses that were successful in this study. More studies on the shelf life of products after irradiation are warranted to fully elucidate sensory and quality responses of treated foods.

4. Conclusions

Irradiation treatments at doses as low as 0.2 kGy effectively inactivate *T. gondii* oocysts on blueberries surfaces with minimal impact on texture, color, or anthocyanin content of treated berries. Since this study examined surface contamination of *T. gondii* on blueberries, the inactivation of the oocysts was not affected by the chemical properties in the product. Therefore, the effective irradiation doses may also be applicable to other berries or produce for decontaminating *T. gondii*.

289   **Acknowledgement**

290   This study was supported by the USDA National Institute of Food and Agriculture, award
291   number 2012-67006-19599.

292

293

Figures:

Figure 1. Cell viability for irradiated blueberry samples inoculated with *Toxoplasma gondii* oocysts. Results were calculated with two samples per treatment conducted in duplicate. Bars for specified treatment not followed by the same letter are significantly different ($p < 0.05$).

Figure 2. The immediate effect of irradiation treatment on total anthocyanins and compression firmness on blueberries. Results represent the mean $\pm$ the standard error. Left axis displays irradiation effect on total anthocyanins (mg/g cyanidin-3-glucoside equivalents (mg/g C3G eq.)) for irradiated blueberries. Right axis displays compression firmness in Newtons (N).

REFERENCES

- Batz, M. B., Hoffmann, S., & Morris, J. G. (2012). Ranking the disease burden of 14 pathogens in food sources in the united states using attribution data from outbreak investigations and expert elicitation. *Journal of Food Protection* 7, 1278-1291.
- Bjoroy, S. Rayyan, T. Fossen, O.M. Andersen (2009). Structural properties of anthocyanins: rearrangement of c-glycosyl-3-deoxyanthocyanidins in acidic aqueous solutions. *Journal of Agricultural Food Chemistry.*, 15, pp. 6668–6677
- CDC, U.S. (2011). Estimates of foodborne illness in the United States.
<http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html>.
- Cole, M., Kasuga, F., Farber, J. M., Anderson, W., Anelich, L., & Buchanan, R. L. 2011. Fruits and fruit products. In *Microorganisms in Foods 8: Use of Data for Assessing Process Control and Product Acceptance* (pp. 177-195). New York: Springer.
- Dubey, J. P. (1996). *Toxoplasma Gondii*. In B. Samuel (Ed.), *Medical Microbiology* (4th ed.). Galveston, Texas: University of Texas Medical Branch at Galveston.
- Dubey, J. P. (2009). General biology. In J. P. Dubey (Ed.), *Toxoplasmosis of Animals and Humans* (pp. 55-64). Boca Raton, Florida: CRC Press.
- Dubey, J. P. (2004). Toxoplasmosis – a waterborne zoonosis. *Veterinary Parasitology*, 126, 57-72.
- Dubey, J. P., Kotula, A. W., Sharar, A., Andrews, C. D., & Lindsay, D. S. (1990). Effect of high-temperature on infectivity of *Toxoplasma-gondii* tissue cysts in pork. *Journal of Parasitology*, 76, 201-204.

325 Dubey, J. P., Thayer, D.W., Speer, C. A., & Shen, S. K. (1998). Effect of gamma irradiation on
 326 unsporulated and sporulated *Toxoplasma gondii* oocysts. *International Journal for*
 327 *Parasitology* 28, 369-375.

328 Dubey, J. P., & Thayer, D. (1994). Killing of different strains of *Toxoplasma-gondii* tissue cysts
 329 by irradiation under defined conditions. *Journal of Parasitology*, 80, 764-767.

330 Ekman, C. C. J., Chiossi, M. F. D., Meireles, L. R., de Andrade, H. F., Figueiredo, W. M.,
 331 Marciano, M. A. M., & Luna, E. J. D. (2012). Case-control study of an outbreak of acute
 332 toxoplasmosis in an industrial plant in the state of Sao paulo, Brazil. *Revista Do Instituto*
 333 *De Medicina Tropical De Sao Paulo*, 54, 239-244.

334 El Said Said, D. (2012). Detection of parasites in commonly consumed raw vegetables.
 335 *Alexandria Journal of Medicine*, 48, 345-352.

336 EPA,U.S.(2014).RadiationProtection:foodsafety.http://www.epa.gov/radiation/sources/food_safety.html .
 337

338 FDA, U.S. (2012). *Irradiation in the production, processing and handling of food*. U.S. Code of
 339 Federal Regulations, Chapter 21, Part 179.

340 Havelaar, A. H., Haagsma, J. A., Mangen, M.-J. J., Kemmeren, J. M., Verhoef, L. P. B., Vijgen,
 341 S. M. C., . . . van Pelt, W. (2012). Disease burden of foodborne pathogens in the
 342 Netherlands, 2009. *International Journal of Food Microbiology*, 156(3), 231-238. doi:
 343 <http://dx.doi.org/10.1016/j.ijfoodmicro.2012.03.029>

344 Hoffmann, S., Batz, M. B., & Morris Jr, J. G. (2012). Annual Cost of Illness and
 345 QualityAdjusted Life Year Losses in the United States Due to 14 Foodborne Pathogens.
 346 *Journal of Food Protection*, 75(7), 1292-1302. doi: 10.4315/0362-028x.jfp- 11-417

348 Kniel, K. E., Lindsay, D. S., Sumner, S. S., Hackney, C. R., Pierson, M. D., & Dubey, J. P.
 349 (2002). Examination of attachment and survival of *Toxoplasma gondii* oocysts on
 350 raspberries and blueberries. *Journal of Parasitology*, 88, 790-793.

351 Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin
 352 pigment content of fruit juices, beverages, natural colorants, and wines by the pH
 353 differential method: collaborative study. *Journal of AOAC International*, 88, 1269-1278.

354 Lee, J. M., Rennaker, C., & Wrolstad, R. E. (2008). Correlation of two anthocyanin
 355 quantification methods: HPLC and spectrophotometric methods. *Food Chemistry*, 110,
 356 782-786.

357 Lindsay, D. S., Holliman, D., Flick, G. J., Goodwin, D. G., Mitchell, S. M., & Dubey, J. P.
 358 (2008). Effects of high pressure processing on *Toxoplasma gondii* oocysts on raspberries.
 359 *Journal of Parasitology*, 94, 757-758.

360 Patras, A. N.P. Brunton, C. O'Donnell, B.K. Tiwari (2010).Effect of thermal processing on
 361 anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends Food*
 362 *Science and Technology* ., 21 , pp. 3–11

363 Saadatnia, G., Haj Ghani, H., Khoo, B. Y., Maimunah, A., & Rahmah, N. (2010). Optimization
 364 of *Toxoplasma gondii* cultivation in VERO cell line. *Tropical Biomedicine*, 27, 125-130.

365 Saftner, R. Polashock, J., Ehlenfeldt, M., Vinyard B., (2008) Instrumental and sensory quality
 366 characteristics of blueberry fruit from twelve cultivars. *Postharvest Biology Technology*.,
 367 49, pp. 19–26

368 Sivapalasingam, S., Friedman, C. R., Cohen, L., & Tauxe, R. V. (2004). Fresh produce: A
 369 growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997.
 370 *Journal of Food Protection*, 10, 2342-2353.

- Tiwari, B.K. O'Donnell, C.P., Cullen P.J.(2009) Effect of non thermal processing technologies on the anthocyanin content of fruit juices *Food Science and Technology*, 20 (2009), pp. 137–145
- Villegas, E. N., Augustine, S. A. J., Villegas, L. F., Ware, M. W., See, M. J., Lindquist, H. D. A., & Dubey, J. P. (2010). Using quantitative reverse transcriptase PCR and cell culture plaque assays to determine resistance of *Toxoplasma gondii* oocysts to chemical sanitizers. *Journal of Microbiological Method*, 81, 219-225.
- Wainwright, K. E., Miller, M. A., Barr, B. C., Gardner, I. A., Melli, A. C., Essert, T., & Conrad, P. A. (2007). Chemical inactivation of *Toxoplasma gondii* oocysts in water. *Journal of Parasitology*, 93, 925-931.
- Ware, M. W., Augustine, S. A. J., Erisman, D. O., See, M. J., Wymer, L., Hayes, S. L., & Villegas, E. N. (2010). Determining UV inactivation of *Toxoplasma gondii* oocysts by using cell culture and a mouse bioassay. *Applied and Environmental Microbiology*, 76, 5140-5147.
- Wu, V. C. H., & Kim, B. (2007). Effect of a simple chlorine dioxide method for controlling five foodborne pathogens, yeasts and molds on blueberries. *Food Microbiology*, 24, 794-800.
- Wu, X. L., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *Journal of Agricultural and Food Chemistry*, 54, 4069-4075.

392 Yannopoulos, S.I., Lyberatos, G., Theodossiou, Valipour, N.M., Tamburrino, A. and Andreas N.
393 Angelakis (2015) Evolution of Water Lifting Devices (Pumps) over the Centuries Worldwide.
394 Water, 7, 5031-5060;
395

Figure 1.

Figure 2.

Table 1. Surface color (Hunter *L**, *a**, and *b**) for blueberries after treatment with irradiation

Treatment (kGy)	<i>L*</i> value	<i>a*</i> value	<i>b*</i> value
0.0	30.72 ± 0.72A	-0.26 ± 0.72A	-5.12 ± 0.26A
0.2	30.35 ± 1.27A	-0.30 ± 0.06A	-4.78 ± 0.57A
0.4	30.28 ± 1.73A	-0.31 ± 0.11A	-5.03 ± 0.55A
0.6	30.24 ± 1.00A	-0.28 ± 0.06A	-4.77 ± 0.29A

^a Each treatment consists of 5 samples, each with 5 berries and each berry had readings on opposing sides.

^b Values within the same column for a specified treatment not followed by the same letter are significantly different (*P* < 0.05).