

Response to Readers' Comments on Published Paper: "Ultraviolet Treatment of Orange Juice to Inactivate *E. coli* O157:H7 as Affected by Native Microflora" by Juan M. Oteiza, Leda Giannuzzi, Noemí Zaritzky [Food and Bioprocess Technology 3-4 (2010) 603-614]

UV Irradiation Dose Corrections

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In this paper, we described that for each assay, 5 ml of inoculated orange juice (with individual *Escherichia coli* O157:H7 strains or with the strain cocktail) was placed in sterile Petri dishes (9 cm in diameter) separately; thus, a layer of 0.7-mm thick was obtained; the distance between the sample and the UV lamp was fixed at 15 cm. A special UV chamber was designed with four UV germicidal, mercury low-pressure lamps (254 nm, UV, Lux 30 W/G30 T8, Philips), located on the top of the chamber. To analyze the effect of the stirring velocity, an orbital shaker with selectable speed was used. This velocity was set at 220 rpm. A ventilation system was included in the chamber to avoid warming of the samples. UV intensity flux or irradiance at 254 nm (I , expressed in milliwatts per square centimeter), exposures times (t , ranging between 0 and 10 min), and the radiation dose (energy, $E=I \times t$, ranging between 0 and 2 J/cm²) were measured using a UV digital radiometer (Vilber Lourmat, model VLX-3 W CE; France).

The radiometer was placed at a similar distance from the UV lamp as the treated juice samples. Prior to usage, the collimated beam apparatus was cleaned and sanitized.

For the UV inactivation treatments, inoculated plates were subjected to different incident doses of UV light.

According to the methodology described in the paper, in each case, we measured the UV intensity at the surface of the sample (incident intensity (I_0) or irradiance at the surface). Therefore, we reported in the paper the incident doses and not the absorbed ones that are obviously lower.

It must be considered that this paper is the continuation of a previous one: "Antimicrobial Efficacy of UV Radiation on *Escherichia coli* O157:H7 (EDL 933) in Fruit Juices of Different Absorptivities" (authors: J.M. Oteiza, M. Peltzer, L. Giannuzzi, and N. Zaritzky, published in the *Journal of Food Protection*, vol. 68, 1, 49–58, 2005). In this previous paper that was cited in the references, we analyzed three types of fruit juices (orange, apple, and multifruit) with different absorptivities under several operating conditions (liquid film thickness, agitation rates). We showed that the higher the absorbance of the medium, the greater the values of D required to inactivate *E. coli* strains by UV. In this previous paper, we differentiated the incident energy from the absorbed one. An equation was developed which relates the fraction of energy absorbed by the system (AEF) with the thickness of the film exposed to UV and the absorptivity coefficient of the juices.

For example, in the case of nonstirred samples, we explained that when electromagnetic radiation of power P_0 impinges on a liquid system, it leads to interactions of photons and absorbent particles, so the transmitted radiation reduces to from P_0 to P . On these grounds, the solution transmittance T is the fraction of incident radiation that is transmitted by the solution, i.e., $T=P/P_0$. The Lambert-Beer law states that

$$P = P_0 \exp(-a \cdot b \cdot c) \quad (1)$$

where a is the molar absorptivity (liters per mole per centimeter), b is the path of the cuvette (sample holder, centimeters), while c is the concentration in solution (moles per liter).

The absorbed energy fraction (AEF) by a sample can be expressed as follows:

$$AEF = (P_0 - P)/P_0 = 1 - \exp(-a \cdot b \cdot c) \quad (2)$$

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where $P - P_0$ is the absorbed energy, and P_0 the incident energy; a is the specific absorptivity, c is the food concentration, and b is the thickness of the exposed film. Equation 2 shows a relationship between AEF and film thickness.

For a given juice concentration the product of a and c was considered constant ($a \cdot c = cte = \alpha$).

In this paper, the calculated values of AEF for the stagnant liquid films of juice with different thickness values (0.7 and 2.8 mm) exposed to UV radiation were reported.

The following table (extracted from Table 4 of the paper “Antimicrobial Efficacy of UV Radiation on *Escherichia coli* O157:H7 (EDL 933) in Fruit Juices of Different Absorptivities” (authors: J.M. Oteiza, M. Peltzer, L. Giannuzzi, and N. Zaritzky, *Journal of Food Protection*, vol. 68, 1, 49–58, 2005) shows the AEF values of the different juices under two conditions of layer thickness with and without stirring.

Absorptivity of fruit juices	Thickness of the stagnant liquid film (mm)	AEF (absorbed energy factor)	D_{TSA} (J/cm ²)	
			ATCC 25922	O157:H7 EDL 933
Orange ($\alpha=0.0715$)	0.7	0.005	0.96	0.99
	2.8	0.02	6.67	6.67
Apple ($\alpha=0.3528$)	0.7	0.02	2.50	2.50
	2.8	0.09	16.67	16.67
Multifruit ($\alpha=0.7230$)	0.7	0.05	5.00	5.26
	2.8	0.18	20.00	20.00

α indicates the absorptivity values

As can be observed in the table, the absorbed UV energy is significantly lower than the incident doses.

Unfortunately, when we sent the final version in the second paper “Ultraviolet Treatment of Orange Juice to Inactivate *E. coli* O157:H7 as Affected by Native Microflora,” we eliminated all these comments in order to shorten the length of the manuscript and to avoid repetitions, because the objective was to remark the effect of yeasts on the absorptivity of the juices.

We agree with Dr. Ankit Patras and Dr. Tatiana Koutchma that the reported doses to reduce 5D *E. coli* in our paper are high, but it must be taken into account that they represented the incident doses, according to the materials and methods section. Perhaps, this concept was not sufficiently clarified in the paper; therefore, these values should be affected by coefficients that consider the different factors you have mentioned, in order to obtain the effective doses.

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