Escherichia coli O157:H7 and *Salmonella* Typhimurium inactivation by the effect of mandarin, lemon, and orange by-products in reference medium and in oat-fruit juice mixed beverage

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Abstract

The antimicrobial capability of three water extracts of citrus peels was evaluated against *S.* Typhimurium and *E. coli* O157:H7 at various concentrations (0.5, 1, 5, 10%) and temperatures (5, 10, 22°C) in a reference medium. The best of them was mandarin by-product, achieving a maximum inactivation level against *S.* Typhimurium (8 $\log_{10}$ cycles) with 5% at 5°C. Also, this by-product had the highest total polyphenol content. Mandarin by-product showed a bactericidal effect in a food matrix also at 5°C (≈2 $\log_{10}$ cycles). All results were adjusted to the Weibull model and the *b* values indicated that the higher concentration of mandarin, the greater the inactivation rate in reference medium, without significant differences between 5 and 10%. Similarly, in the food matrix, the inactivation rate of *S.* Typhimurium was higher when the mandarin by-product was added. Therefore, the mandarin by-product could be used as a control measure of *S*. Typhimurium in pasteurized products, which are stored under refrigeration.

Keywords: citrus by-products; new ingredients; antimicrobials; bactericidal concentration

1. Introduction

Citrus is the largest fruit crop worldwide, with an annual production of approximately 100 million tons. The main world producers are Brazil, the USA and Mediterranean countries (Djilas, 2009; Ghafar et al., 2010). The industrial production of juices and other citrus derivatives generates approximately 15 million tons of citrus waste a year worldwide, which mainly consists of peel, seeds, and the fruit pulp. Citrus waste is usually consigned to landfill or incineration, which generates negative effects on the environment and a cost to the producers (O'Shea et al., 2012).

This valueless citrus waste can be considered as a renewable source of raw material whose use in various industrial fields could have a double benefit, economic and technological, as a result of its valorization (Schieber et al., 2001; Martín-Luengo et al., 2011). Since 2010 generalized agri-food by-product valorization has been a European Union requirement (EUROSTAT, 2010) and many research studies nowadays are focused on recovering, revaluing, and recycling these by-products. One way of valorizing these by-products is the formulation of new products with added nutritional value. Citrus by-products are rich in functional compounds such as carotenoids and flavonoids, among others (O'Shea et al., 2012), whose antioxidant, anticarcinogenic, antiviral, and anti-inflammatory properties are well known. Citrus derivative compounds have an important nutritional and flavoring value, and an antimicrobial capability has also been attributed to some of them, mainly due to ferulic acid, hydrocinnamic acid, yaniding glucoside, hisperidin, vitamin C, carotenoid, and naringin (Ghafar et al., 2010). In this sense, they could be used like natural antimicrobials to control the growth of foodborne pathogens, replacing the chemical compounds which are used currently. Also, they could be used as an additional control measure of the microbial growth in situations of cold chain breakdown in pasteurized food that is stored in refrigeration (Sanz-Puig et al., 2015).

In this context, the aim of this study was to evaluate the antimicrobial effect of water extracts of by-products of citrus fruits – mandarin, orange, and lemon – against two of the foodborne pathogens of most concern that are found in low-acid beverages: *Salmonella enterica* serovar Typhimurium and *Escherichia coli* O157:H7.

2. Material and Methods

2.1 Microbiology

Pure cultures of *S.* Typhimurium (CECT 443) and *E. coli* O157:H7 (CECT 5947) were provided freeze-dried by the Spanish Type Culture Collection. Both cultures were rehydrated with 10 mL of Tryptic Soy Broth (TSB) (Scharlab Chemie, Barcelona, Spain). After 20 minutes, the rehydrated culture was transferred to 500 mL of TSB and incubated at 37°C with continuous shaking at 200 rpm for 14 hours to obtain cells in a stationary growth stage. The cells were centrifuged twice at 4000 × g at 4°C for 15 minutes and then resuspended in TSB. After the second centrifugation, the cells were resuspended in 20 mL of TSB with 20% glycerol, and then dispensed in 2-mL vials with a final concentration of 10^8 colony forming units per milliliter (CFU/mL). The 2-mL samples were immediately frozen and stored at –80°C until needed for the kinetic inactivation studies.

2.2 Citrus by-products

Dehydrated peel residues from mandarin (*Citrus reticulata*), orange (*Citrus sinensis*) and lemon (*Citrus limon*) were provided from primary production (Indulleida, S.A.). Each raw by-product was tested to screen its bacteriological quality. The bacteriological analysis determined the presence/absence of microbial contamination with *Listeria monocytogenes* and *Bacillus cereus* (Gram-positives), or *E. coli* O157:H7 and *S.* Typhimurium (Gram-negatives), and was carried out according to the procedures described by Aycicek et al. (2006). No samples studied presented contamination with any of the microorganisms tested.

2.3 Total Phenolic Compounds

The total phenol content of the citrus by-products was determined spectrophotometrically according to the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965). Gallic acid calibration standards with concentrations of 0, 100, 200, 300, 400, 500, 600, 700, 800, and 1000 ppm were prepared. Three mL of sodium carbonate solution (2% (w/v)) (Sigma-Aldrich Co. LLC, USA) and 100 µL of Folin-Ciocalteu reagent (1:1 (v/v)) (Sigma-Aldrich Co. LLC, USA) were added to an aliquot of 100 µL from each gallic acid standard (Sigma-Aldrich Co. LLC, USA) or sample tube. The mixture was shaken and allowed to stand at room temperature in the dark for 1 h. Absorbance was measured at 750 nm using a Lan Optics Model PG1800 spectrophotometer (Labolan, Spain), and the results were expressed as mg of gallic acid equivalents (GAE)/L.

2.4 Antimicrobial assay

Buffered peptone water (Scharlab Chemie, Barcelona, Spain) (0.1% (w/v)) was used as a reference substrate in the present study. For the assessment of citrus by-product antimicrobial capability, 1 mL of each vial of stock culture was added to reference substrate at a final concentration of 10^7 CFU/mL. The inoculated medium (buffered peptone water) was supplemented with dehydrated peel residues at different concentrations (0.5, 1, 5, and 10% (w/v)). All the samples were then incubated at different temperatures (5, 10, and 22°C). At regular time intervals (hours), the cell suspension for each sample was evaluated by plate count in Tryptic Soy Agar (TSA) (Scharlab Chemie, Barcelona, Spain) after serial dilution with 0.1% (w/v) buffered peptone water. The plates were incubated at 37°C for 24 hours. Each dilution was plated in duplicate. The experiments were carried out in triplicate and the plate counts were used for CFU/mL enumeration.

A second set of experiments was conducted. The most effective antimicrobial of the three tested in the reference medium was evaluated against *S.* Typhimurium in various formulated beverages.

Finally, in order to compare the results, the behavior of both microorganisms under exposure to citrus by-product was characterized by estimating the minimal inhibitory concentration (MIC), being the lowest concentration of antimicrobial substance that is able to inhibit microbial growth (Guillier et al., 2007).

Also, the minimal bactericidal concentration (MBC) was estimated, being the lowest concentration of antimicrobial substance that is able to exert a bactericidal effect against the microorganism under study (Bär et al., 2009).

2.5 Food Matrix

The antimicrobial potential of the most bactericidal citrus by-product was tested against both pathogens in complex food matrices. Firstly, an oat beverage (OB) was used in this set of experiments. The beverage used was supplemented with the most effective citrus by-product and compared with the non-supplemented beverage. The concentration of the by-product was the minimum bactericidal concentration (MBC), and the incubation temperature was 5°C, a typical temperature for storage of beverages of this kind. Secondly, an oat beverage containing 32.5% papaya, 10% mango, and 7.5% orange (OB-FM) was used. As in the case of the oat beverage, this beverage was supplemented with the most effective antimicrobial by-product using the minimum bactericidal concentration (MBC). The results were compared with those obtained in the non-supplemented OB-FM beverage.

The food matrices considered, OB (supplemented/not supplemented) and OB-FM (supplemented/not supplemented with the most bactericidal by-product), were inoculated with an initial microbial population of 10^8 CFU/mL. The bacterial growth/death during refrigerated storage was monitored by means of viable cell counts.

2.6 Modeling of microorganism inactivation

The microbial behavior was fitted to a Weibull equation (Peleg and Cole, 1998) to obtain a mathematical description of the kinetics of bacterial inactivation by the citrus by-product:

$$\log_{10}\left(S(t)\right) = -b \times t^n \tag{1}$$

where *t* is the time (hours), S is the survival fraction, i.e., the quotient between the cell concentration at time *t* (N_t) (CFU/mL) and the initial cell concentration (N_0) (CFU/mL); *b* is the scale factor and *n* is the form factor.

2.7 Data analysis and model evaluation

The statistical analysis was performed with STATGRAPHICS Centurion XV (version 15.1.03; STATGRAPHICS, Warrenton, VA).

This analysis included average and standard deviation calculations for the three repetitions and an ANOVA analysis to test significant differences depending on incubation conditions. The goodness of fit of the model was assessed by using the adjusted regression coefficient (*adjusted*-R^2) (López et al., 2004).

3. Results and Discussion

3.1 Antimicrobial capacity of Citrus by-products against S. *Typhimurium*

The antimicrobial effect of the mandarin, orange, and lemon by-products was evaluated against *S.* Typhimurium cells during 96 hours of incubation at 5 and 10°C and 24 hours of incubation at 22°C. Figure 1 shows the log cycle reduction achieved for each combination.

With regard to the effect of temperature, *S.* Typhimurium growth was inhibited in non-supplemented reference medium (0%) with refrigerated incubation of 5°C, while at 10°C detectable growth was observed after 96 hours, and it was higher at 22°C. Therefore it can be concluded that low temperature acts as an effective bacterial proliferation barrier against *S.* Typhimurium, which is in agreement with the findings of other authors (Okada et al., 2013).

In general, all by-products tested reduced the microbial load of *S.* Typhimurium regardless of the incubation temperature, with a maximum reduction very close to 8 $\log_{10}$ cycles at 5% and 10% mandarin by-product and 5 and 10°C incubation temperature. We note that mandarin was the most effective by-product, followed by orange and lemon.

With regard to the by-product concentration, only 5 and 10% of orange and lemon by-products could be considered as an additional control measure for *S.* Typhimurium in the case of a cold chain break (22°C), at least for 24 hours. In contrast, for mandarin by-product, all concentrations tested could be used. In the case of temperature abuse (10°C), 5 and 10% of by-product could also be considered as an additional control measure for this microorganism, at least for 96 hours, although in orange by-product no significant differences ($p \leq 0.05$) were observed among the concentrations studied.

An ANOVA analysis concluded that both incubation temperature and by-product concentration had a significant impact ($p \leq 0.05$) on *S.* Typhimurium cell survival. As can be seen in Figure 1, at all temperatures an increase in citrus by-product concentration was accompanied by greater microorganism growth inhibition or inactivation. However, no significant differences were observed between inactivation levels achieved when citrus by-product was added to the medium at 5–10%, with inactivation levels very close to 8 $\log_{10}$ cycles at incubation temperatures of 5 and 10°C in samples with mandarin by-product.

The antimicrobial potential of the by-products studied could be particularly relevant under the concept of hurdle barriers, acting as an additional measure to control bacterial proliferation in situations of abuse temperature (10°C) or in the case of cold chain breakdown (22°C) in pasteurized food products which must be storage at refrigeration temperatures. They can be added to this kind of products (fruit or vegetable creams or beverages) like an ingredient and control the microbial growth during their storage period. However, these by-products have a low but characteristic taste and odour that could not be accepted by the consumers at high concentrations. Therefore, is important to carry on a sensorial study with the aim to find the concentration of by-product with an antimicrobial capability and sensorial acceptance (Valero & Giner, 2006) and the food products where it could be added.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) for each citrus by-product in relation to incubation temperature were calculated (Table 1). *S.* Typhimurium is highly sensitive to contact with citrus by-products, with very low MIC and MBC values (0.5%). The microbial sensitivity of *S.* Typhimurium depends on both the temperature and the citrus by-product type ($p < 0.05$). The lowest MBC was obtained for mandarin at 5 and 22°C; while lemon and orange required a smaller MBC than mandarin to be effective against *S.* Typhimurium when the incubation temperature was 10°C.

Generally, the MBC at refrigeration temperatures was lower than at room temperature (22°C). This may be because refrigeration temperatures have a bacteriostatic capacity and exert a synergistic or additive effect with the by-product concentration. Other authors have shown the bacteriostatic capacity of refrigeration temperatures and have attributed it to a stress response mechanism that is activated in microorganisms at low temperatures (Shapiro and Cowen, 2012).

3.2 Antimicrobial capacity of Citrus by-products against E. coli *O157:H7*

The results for the effect of the citrus by-products on *E. coli* O157:H7 are shown in Figure 2.

As can be seen, low temperature (5°C) inhibited *E. coli* O157:H7 cell growth in reference medium (0% by-product), while at 10 (abuse of temperature) and 22°C (cold chain break) the microorganism was able to grow. Focusing on the effect of by-product concentration, 5 and 10% mandarin and orange by-product had a bactericidal effect (≥ 0.5 log_{10} cycles), reducing *E. coli* O157:H7 counts by a maximum of 1.5 log_{10} cycles. The effect of 5 and 10% concentrations on the bacteriostatic or bactericidal effect at temperatures other than 5°C depended on the citrus by-product used. Concentrations lower than 5% appear to have a bacteriostatic effect, slowing down growth of the microorganisms. Note that at 10°C *E. coli* O157:H7 started to grow and addition of the mandarin by-product showed a bacteriostatic capacity. In contrast, addition of the orange and lemon by-products did not have any antimicrobial (bacteriostatic or bactericidal) effect at this temperature. At 22°C, the by-products studied had a bacteriostatic effect against *E. coli* O157:H7 when they were added at 5% (w/v), and addition of mandarin by-product at 10% (w/v) had a bactericidal effect, achieving a maximum reduction of 1.6 log_{10} cycles.

The mandarin by-product also showed the highest antimicrobial potential against *E. coli* O157:H7, with reductions of 1.3 and 1.6 log_{10} cycles at 5 and 22°C, respectively. The orange and lemon by-products achieved a bactericidal effect, with reductions ranging from 0.5 to 1 log_{10} cycles at refrigeration temperatures, and both exerted a bacteriostatic effect at 22°C.

It is important to note that the effect of the by-products depended on the microorganism tested and the polyphenol structure (Taguri et al., 2004; Daglia, 2011). In our case, S. Typhimurium was more sensitive than E. coli O157:H7 to the various by-products used. This might indicate that each antimicrobial could be specific against a particular microorganism or group of microorganisms.

An ANOVA analysis of data for *E. coli* O157:H7 revealed that for all the by-products studied both incubation temperature and by-product concentration had a significant influence on the antimicrobial activity against *E. coli* O157:H7 ($p < 0.05$), achieving the highest antimicrobial effect by 5 and 10% by-product addition, without significant differences between them.

Table 2 shows the MIC and MBC of the citrus by-products against *E. coli* O157:H7 for each combination of the factors (temperature – concentration) tested. The MIC values are 0.5% at all the temperatures studied, and the MBC values are between 1 and 5%, both being influenced by the incubation temperature and the type of citrus by-product added.

The mandarin by-product had a bactericidal effect at 5°C, a bacteriostatic effect at 10°C, and both at 22°C. However, although the orange and lemon by-products have the same MIC and MBC values as the mandarin by-product at 10 and 22°C, they showed a lower antimicrobial capacity expressed as $\log_{10}$ cycle reduction. Therefore, under the conditions studied, it is possible to conclude that *E. coli* O157:H7 has less sensitivity to the citrus by-products studied than *S.* Typhimurium.

It is well known that the antimicrobial effect of many natural products in a real or buffered medium is influenced by environmental factors (e.g., pH and temperature conditions), the concentration of the natural ingredient, and the sensitiveness of the microbe (e.g., strain, virulence) (Bajpai, 2012).

Table 3 shows the pH values for the citrus by-products tested at concentrations of 5 and 10%. Although it has traditionally been accepted that pH plays an important part in inhibiting cellular activity, the table shows that the citrus by-product with the lowest pH value is lemon, while the by-product with the best antimicrobial effect against the microorganisms under study is mandarin. This result appears to indicate that pH is not the most important factor that influences citrus by-product antimicrobial activity.

3.3 Polyphenol concentration of Citrus by-products

The bacteriostatic and bactericidal capacities of citrus by-products could be significantly influenced by their composition, mainly because of their polyphenol content. Numerous studies show that they have many bioactive compounds such as polyphenols, including ferulic acid, hydrocinnamic acid, cyaniding glucoside, hisperidin, carotenoid, and naringin, in their peel and seeds, which have antioxidant and antimicrobial properties (Ghafar et al., 2010). Table 4 shows the polyphenol content measured for each citrus by-product under study. As can be seen in the table, the mandarin by-product has the highest total polyphenol content, followed by orange and then lemon. In this case, the total polyphenol content coincides with the antimicrobial capacity of the by-products: the citrus by-product with the highest polyphenol content, mandarin, is the one with the greatest antimicrobial capacity, followed by the orange and lemon by-products. Therefore we can conclude that polyphenol content may be directly related to antimicrobial activity, in accordance with other studies (Devi et al., 2008).

3.4 Mathematical modeling of S. *Typhimurium and* E. coli *O157:H7 inactivation*

The experimental curves obtained for *S.* Typhimurium and *E. coli* O157:H7 were fitted to a Weibull distribution function, owing to its simplicity and robustness for describing inactivation kinetics (De Oliveira et al., 2013).

The results of the fitting are shown in Tables 5 and 6. The *b* value is related to inactivation rate: the higher the *b* value, the faster the microorganism dies. The Weibull kinetic *b* values for *S.* Typhimurium (Table 5) increase with higher by-product concentrations, achieving the maximum inactivation rate at 5% by-product concentration, without significant differences (>0.05) between the *b* values at 5 and 10% by-product concentration.

The same pattern occurs in the *E. coli* O157:H7 inactivation kinetics. As can be seen in Table 6, at lower by-product concentrations the *b* values are close to 0 or negative, owing to microorganism growth. However, at higher citrus by-product concentrations the *b* value increases, without significant differences between 5 and 10% (w/v) addition.

Therefore the concentration of citrus by-product added affects the inactivation rate of the two Gram-negative microorganisms studied. In contrast, there does not appear to be a relationship between incubation temperature and *b* value, and, therefore, with the rate of microorganism inactivation.

3.5 Antimicrobial potential of mandarin by-product incorporated in an oat-based beverage

According to the results in the previous sections, mandarin (MND) had the highest antimicrobial potential among the citrus by-products studied in reference medium.

Table 7 shows the inactivation levels reached in *S.* Typhimurium and *E. coli* O157:H7 in oat beverage (OB) supplemented or not supplemented with mandarin during the refrigerated storage period of 144 hours at 5°C. Although temperature produces some log reductions in the microbial load, an additive effect can be attributed to the mandarin by-product added to the real beverages, producing an additional reduction for *S.* Typhimurium of 0.47 $\log_{10}$ cycles when MND was incorporated in OB and 0.68 $\log_{10}$ cycles when MND was added to oat-based beverage with fruit juice mixture (OB+FM);

and for *E. coli* O157:H7 additional reductions close to 1.18 log_{10} cycles were achieved when MND was incorporated in OB, and 0.65 log_{10} cycles when MND was added to OB+FM. Although MND had higher effectiveness against *S.* Typhimurium in reference medium, *E. coli* O157:H7 was more sensitive when MND was added to the food matrices studied. It can be observed that the inactivation levels achieved for both microorganisms in OB+FM were significantly ($p \leq 0.05$) higher than those achieved in OB. Some research studies have shown that many fruits are rich in bioactive compounds with antioxidant properties, such as polyphenols, which could also have additional antimicrobial properties against foodborne pathogens (Ghasemi et al., 2009; Mandalari et al., 2007).

According to the results obtained, the bactericidal effect of mandarin on both microorganisms was higher in reference medium than in food matrix. When the mandarin by-product was added to a real matrix, its antimicrobial effectiveness against S. Typhimurium was 75% less than when it was added to the reference medium. The interference of the real substrate was remarkable in the case of the *S.* Typhimurium growth/death pattern under refrigeration using OB as the food matrix. The addition of MND (5% (w/v)) in reference medium resulted in a reduction of 8 log_{10} cycles for *S.* Typhimurium, while incorporation of this by-product in OB only produced a reduction close to 1 log_{10} cycle under the same time and temperature storage conditions (96 h, 5°C). Several authors attribute to food matrix complexity a protective effect that reduces the effectiveness of many control treatments (Gutierrez et al., 2008). The protective effect of a lipid-rich substrate such as oat milk could affect the antimicrobial potential of mandarin against *S.* Typhimurium (Di Pascua et al., 2006).

The addition of a papaya, mango, and orange juice mixture to the beverage studied significantly increased the inactivation values at each storage point recorded for both microbial populations. After the complete storage period, *S.* Typhimurium inactivation was almost doubled (increasing from 0.74 log_{10} cycles in OB to 1.25 in OB+FM) by the additional effect of the fruit juices. This may be because mango, orange, and papaya are fruits rich in bioactive substances such as polyphenol compounds (Tomás-Barberán and Espín, 2001), which might produce an antimicrobial effect against the microorganisms studied. Also, the acid pH of the beverage (pH 4.6) might contribute to the antimicrobial effect shown when the fruit juice mixture was added. The supplementation of OB+FM with 5% (w/v) MND increased the final *S.* Typhimurium inactivation level to a maximum of 1.85 $\log_{10}$ cycles compared with the 1.12 $\log_{10}$ cycles achieved in OB+MND, and it increased the maximum *E. coli* O157:H7 inactivation level to 2.22 $\log_{10}$ cycles compared with the 2.01 $\log_{10}$ cycles achieved in OB+MND.

3.6 Mathematical modeling of antimicrobial effect of mandarin by-product addition in an oat-based beverage

The results obtained for microbial inactivation in the oat-based beverage and oat-based beverage with fruit juice mixture, both supplemented/not supplemented with mandarin by-product addition, were fitted to a Weibull distribution function and their kinetic parameters were obtained. The *b* and *n* values obtained are shown in Table 8. In all cases the *n* values are below 0, indicating a concave survival pattern for the microorganisms studied in the beverage. With regard to the scale factor, the *b* values in the fruit juice mixture were higher than those obtained in the oat beverage, indicating the influence of the juice mixture on the microbial inactivation response. The addition of mandarin increased inactivation rates in both OB and OB+FM, with a maximum of 0.571 ± 0.006 for *S.* Typhimurium inactivation and 0.802 ± 0.026 for *E. coli* O157:H7 inactivation in OB+FM supplemented with mandarin by-product.

4. Conclusions

In conclusion, the three citrus by-products under study showed an antimicrobial effect against *S.* Typhimurium. The maximum reduction level was achieved by the mandarin by-product, followed by the orange and lemon by-products.

The same order can be observed in their polyphenol content, so there may be a relationship between the polyphenol content of the citrus by-products and their antimicrobial activity.

Also, the mandarin by-product was able to exert an antimicrobial effect both on a reference medium (8 $\log_{10}$ cycles for *S.* Typhimurium and 1.6 $\log_{10}$ cycles for *E. coli* O157:H7) and on a real food matrix, an oat-based beverage supplemented/not supplemented with a fruit juice mixture (≈2 $\log_{10}$ cycle reductions for *S.* Typhimurium and *E. coli* O157:H7). Therefore this by-product could be used as an ingredient for technological purposes owing to its potential to act as an additional control measure inhibiting bacterial proliferation, e.g., in pasteurized foods, which have limited refrigerated storage.

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Figure Captions

Figure 1. Inactivation levels (Log_{10} (N_f/N_0)) of *S.* Typhimurium in contact with various (0, 0.5, 1, 5, 10%) citric by-products concentrations: mandarin (a), orange (b), and lemon (c) in buffered peptone water, incubated at different temperatures (5, 10, and 22°C).

Figure 2. Inactivation levels (Log_{10} (N_f/N_0)) of *E. coli* O157:H7 in contact with various (0, 0.5, 1, 5, 10%) citric by-product concentrations: mandarin (a), orange (b), and lemon (c) in buffered peptone water, incubated at different temperatures (5, 10, and 22°C).

Table 1

Table 1. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for *S.* Typhimurium in the conditions tested. No significant effects (–).

***S.* Typhimurium**			
Temperature (°C)	**By-product**	**MIC (%)**	**MBC (%)**
5	Mandarin	–	0.5
	Orange	–	1
	Lemon	–	1
10	Mandarin	0.5	5
	Orange	–	0.5
	Lemon	–	0.5
22	Mandarin	–	0.5
	Orange	0.5	5
	Lemon	0.5	5

Table 2

Table 2. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for *E. coli* O157:H7 in the conditions tested. No significant effects (–).

***E. coli* O157:H7**			
Temperature (°C)	***By-product***	***MIC (%)***	***MBC (%)***
5	*Mandarin*	–	*5*
	Orange	–	*1*
	Lemon	–	*5*
10	*Mandarin*	*0.5*	–
	Orange	*0.5*	*5*
	Lemon	–	*5*
22	*Mandarin*	*0.5*	*5*
	Orange	*0.5*	–
	Lemon	–	*5*

Table 3

Table 3. pH values measured for mandarin, orange, and lemon by-products at concentrations of 5 and 10%.

	Mandarin		**Orange**		**Lemon**	
	5%	10%	5%	10%	5%	10%
pH	4.39±0.02	4.24±0.01	4.85±0.04	4.54±0.02	3.92±0.06	3.77±0.06

Table 4

Table 4. Total polyphenol content in by-product extracts.

Citrus by-product	**Polyphenol content (mg gallic acid/L)**
Mandarin 10%	5111.50 ± 201.93
Orange 10%	4809.72 ± 287.47
Lemon 10%	4600.00 ± 20.00

Table 5

Table 5. Weibull kinetic values for *S.* Typhimurium inactivation under the citrus by-product effect at various concentrations (% (w/v)) and temperatures (°C).

% By-product			0%	0.5%	1%	5%	10%
MANDARIN	5 °C	b	0.013±0.017	0.008±0.004	0.132±0.077	1.019±0.118	0.915±0.126
		n	0.804±0.434	1.277±0.144	0.776±0.148	0.420±0.027	0.445±0.040
		R^2	0.904	0.998	0.956	0.965	0.957
	10 °C	b	-0.005±0.054	-0.103±0.153	-0.007±0.022	1.935±0.100	1.668±0.151
		n	0.285±0.159	0.580±0.668	0.558±0.174	0.273±0.010	0.333±0.019
		R^2	0.908	0.905	0.913	0.951	0.958
	22 °C	b	-0.005±0.001	0.036±0.024	0.005±0.004	0.428±0.026	0.300±0.001
		n	1.796±0.053	1.0395±0.265	1.774±0.313	0.510±0.030	0.554±0.012
		R^2	0.948	0.952	0.979	0.979	0.960
ORANGE	5 °C	b	0.009±0.011	0.004±0.005	0.068±0.045	0.350±0.009	0.285±0.010
		n	0.804±0.362	1.839±0.874	1.226±1.274	0.512±0.011	0.561±0.012
		R^2	0.928	0.948	0.960	0.940	0.941
	10 °C	b	-0.098±0.139	0.349±0.141	0.409±0.001	0.303±0.036	0.008±0.005
		n	0.814±1.035	0.387±0.081	0.301±0.013	0.382±0.057	1.178±0.153
		R^2	0.905	0.941	0.951	0.901	0.959
	22 °C	b	-0.005±0.001	-0.014±0.009	-0.034±0.005	0.414±0.009	0.421±0.019
		n	1.796±0.053	0.674±0.200	0.337±0.464	0.301±0.012	0.294±0.020
		R^2	0.948	0.961	0.949	0.964	0.937
LEMON	5 °C	b	0.026±0.012	0.011±0.011	0.057±0.015	0.007±0.004	0.033±0.030
		n	0.418±0.183	0.784±0.175	0.519±0.086	1.090±0.125	0.798±0.223
		R^2	0.940	0.953	0.931	0.935	0.948
	10 °C	b	-0.039±0.039	0.023±0.011	0.027±0.012	0.030±0.026	0.003±0.004
		n	0.458±0.130	0.570±0.142	0.608±0.155	0.701±0.131	1.265±0.396
		R^2	0.934	0.910	0.913	0.951	0.942
	22 °C	b	-0.005±0.001	-0.014±0.009	-0.041±0.004	0.478±0.113	0.384±0.040
		n	1.796±0.053	0.674±0.200	0.170±0.228	0.320±0.092	0.416±0.057
		R^2	0.948	0.961	0.954	0.941	0.916

* The *b* value is negative when the microorganism grows and positive when the microorganism dies.

Table 6

Table 6. Weibull kinetic values for *E. coli* O157:H7 inactivation under the citrus by-product effect at various concentrations (% (w/v)) and temperatures (°C).

% By-product			0%	0.5%	1%	5%	10%
MANDARIN	5 °C	b	0.017±0.008	0.027±0.013	0.033±0.011	0.107±0.016	0.090±0.010
		n	0.561±0.067	0.548±0.230	0.855±0.074	0.613±0.044	0.652±0.018
		R^2	0.925	0.921	0.928	0.938	0.931
	10 °C	b	-0.001±0.001	-0.009±0.016	-0.027±0.037	-0.001±0.001	-0.018±0.006
		n	1.460±0.284	0.663±0.497	0.859±0.561	3.796±0.968	0.344±0.230
		R^2	0.960	0.949	0.955	0.930	0.953
	22 °C	b	-0.036±0.002	-0.015±0.021	-0.116±0.037	0.358±0.178	0.291±0.036
		n	1.138±0.026	1.798±0.912	0.180±0.253	0.450±0.188	0.544±0.041
		R^2	0.973	0.921	0.953	0.942	0.912
ORANGE	5 °C	b	0.005±0.006	0.001±0.001	0.014±0.019	0.001±0.001	0.001±0.002
		n	1.013±0.547	1.385±0.501	1.849±0.470	1.843±0.051	1.532±0.569
		R^2	0.941	0.945	0.965	0.957	0.921
	10 °C	b	-0.001±0.001	0.029±0.028	0.033±0.007	0.023±0.009	0.079±0.013
		n	1.457±0.280	0.403±0.239	0.437±0.035	0.559±0.130	0.438±0.060
		R^2	0.960	0.966	0.939	0.963	0.921
	22 °C	b	-0.036±0.002	-0.038±0.010	-0.051±0.057	0.020±0.028	0.029±0.069
		n	1.138±0.026	1.012±0.071	0.647±0.154	1.078±1.265	0.347±0.490
		R^2	0.973	0.922	0.970	0.939	0.925
LEMON	5 °C	b	0.017±0.006	0.008±0.005	0.017±0.011	0.003±0.003	0.001±0.001
		n	0.526±0.049	0.785±0.091	0.349±0.212	1.137±0.257	1.485±0.007
		R^2	0.935	0.913	0.939	0.927	0.915
	10 °C	b	-0.001±0.001	0.038±0.029	0.014±0.019	0.032±0.018	0.839±0.121
		n	1.378±0.168	0.460±0.132	0.808±0.337	0.669±0.093	0.037±0.038
		R^2	0.952	0.933	0.925	0.935	0.985
	22 °C	b	-0.036±0.002	-0.018±0.018	-0.019±0.017	0.035±0.001	0.001±0.001
		n	1.138±0.026	0.961±0.645	0.769±0.344	0.672±0.094	2.905±1.919
		R^2	0.973	0.961	0.954	0.923	0.965

* The *b* value is negative when the microorganism grows and positive when the microorganism dies.

Table 7

Table 7. Inactivation levels (log_{10} cycles) achieved in the food matrices studied for both *S.* Typhimurium and *E. coli* O157:H7 by the intervention of mandarin (MND) by-product added at MBC 5% during a refrigerated storage period of 144 h at 5 °C.

Microorganism	Storage time (h)	OB	OB+MND	OB+FM	OB+FM+MND
E. coli O157:H7	0	0	0	0	0
	24	-0.10±0.00	-0.92±0.05	-0.91±0.05	-1.75±0.12
	48	-0.15±0.04	-0.96±0.04	-0.96±0.07	-1.92±0.06
	96	-0.72±0.06	-1.12±0.08	-1.06±0.05	-1.73±0.06
	144	-0.83±0.06	-2.01±0.13	-1.57±0.07	-2.22±0.23
S. Typhimurium	0	0	0	0	0
	24	-0.10±0.00	-0.77±0.03	-0.59±0.02	-1.20±0.11
	48	-0.15±0.02	-0.94±0.02	-0.64±0.05	-1.32±0.07
	96	-0.48±0.01	-0.98±0.05	-0.85±0.05	-1.54±0.06
	144	-0.65±0.06	-1.12±0.08	-1.17±0.06	-1.85±0.10

OB: Oat beverage; OB+MND: Oat beverage supplemented with 5% (w/v) mandarin; OB+FM: Oat beverage and fruit juice (papaya, mango, and orange) mixture; OB+FM+MND: Oat beverage and fruit juice mixture supplemented with 5% (w/v) mandarin.

Table 8

Table 8. Weibull kinetic parameters of *E. coli* O157:H7 and *S.* Typhimurium inactivation in Oat beverage and Oat beverage – fruit juice mixture when supplemented/not supplemented with 5% (w/v) mandarin by-product under refrigerated storage (144 h, 5 °C).

Beverage		OB	OB+MND	OB+FM	OB+FM+MND
S. Typhimurium	b	0.014±0.003	0.461±0.015	0.137±0.002	0.571±0.006
	n	0.746±0.012	0.179±0.022	0.419±0.025	0.219±0.011
	Adj-R^2	0.903	0.968	0.962	0.983
	RMSE	0.071	0.051	0.055	0.002
E. coli O157:H7	b	0.018±0.003	0.121±0.011	0.261±0.001	0.802±0.026
	n	0.767±0.025	0.541±0.023	0.325±0.031	0.221±0.021
	Adj-R^2	0.946	0.887	0.915	0.993
	RMSE	0.091	0.062	0.022	0.001

OB: Oat beverage; OB+MND: Oat beverage supplemented with 5% (w/v) mandarin; OB+FM: Oat beverage and fruit juice (papaya, mango, and orange) mixture; OB+FM+MND: Oat beverage and fruit juice mixture supplemented with 5% (w/v) mandarin.

Figure 1

Figure 1.

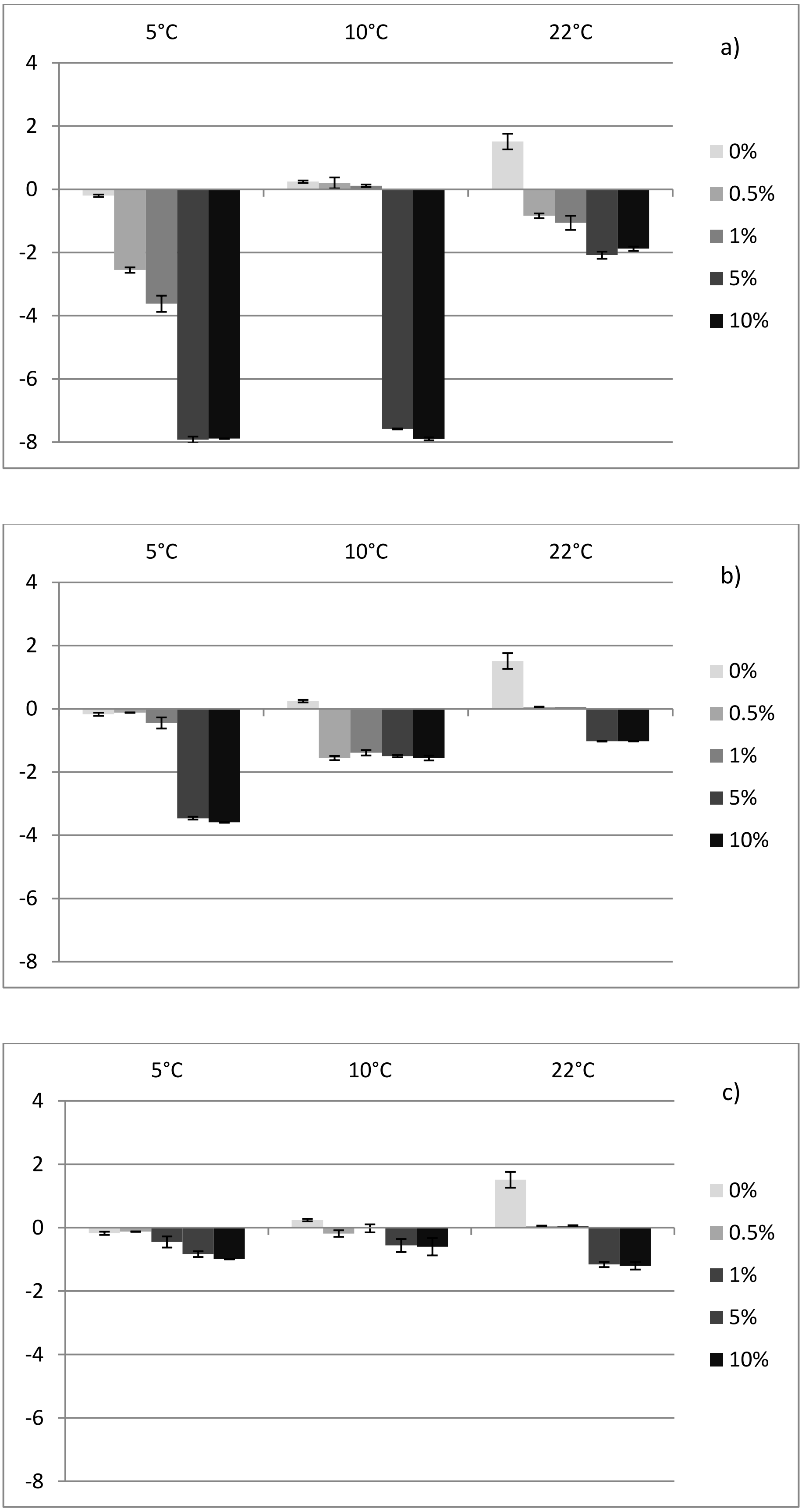

Figure 2

Figure 2.