Journal of Food Process Engineering

Journal of Food Process Engineering ISSN 1745-4530

EXPERIMENTAL EVALUATION OF A NEW THERMAL PROCESS FOR MICROORGANISMS INACTIVATION

MARCO CAMMALLERI[1,3], EMILIANO PIPITONE[1], TERESA RUBINO[1], LAURA SAPORITO[2] and CATERINA MAMMINA[2]

[1]Dipartimento di Ingegneria Chimica, Gestionale, Informatica, Meccanica, Università degli studi di Palermo, viale delle Scienze ed. 8, Palermo 90128, Italy

[2]Dipartimento di Scienze per la Promozione della Salute e Materno Infantile G. D'Alessandro, Università degli studi di Palermo, via del Vespro 129, 90127, Palermo, Italy

[3]Corresponding author.
TEL: +3909123897256;
FAX: +3909123860672;
EMAIL: marco.cammalleri@unipa.it

Received for Publication October 30, 2014
Accepted for Publication November 12, 2014

doi:10.1111/jfpe.12175

ABSTRACT

A new thermal process for the inactivation of microorganisms in beverages has been studied and is presented in this paper. The treatment, not yet studied in the scientific literature, mainly consists of a thermal shock characterized by temperature increases up to 30°C/s, with final temperatures up to 65°C. This study presents the first experimental results obtained by the application of the new thermal treatment, with different combinations of the process parameters (rate of temperature rise, final temperature and holding time), on separate suspensions of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua* and *Candida albicans*. The required rapid temperature increase has been performed by means of a properly designed test bench. The treatment proved to be effective against all the microorganisms tested, obtaining a load reduction greater than 5 Log units. Compared with the commonly employed thermal treatments for microorganisms inactivation, this new process is characterized by a very interesting combination of a short duration (few seconds) and a low final temperature, which makes it particularly attractive for the application in the beverage industry.

PRACTICAL APPLICATIONS

Nowadays, most liquid foods, such as beverages, milk, fruit and vegetables juices, are pasteurized by high-temperature/short-time treatments and then bottled or packaged under rigidly maintained sterile conditions. Thanks to the very short duration of this new treatment and to its relatively low maximum temperature (65°C), which does not endanger the plastic materials commonly employed for bottles packaging, its online application in the filling process could be conveniently considered: this would avoid the use of an aseptic environment and the risk of the post-processing contamination. Moreover, a final temperature as low as 65°C would also preserve the sensorial and nutritional features of the beverage, which are usually deteriorated by the common high temperature treatments, and would be in agreement with the present-day energy-saving policy.

INTRODUCTION

Food and drinks are routinely submitted to pasteurization processing to warrant their safety, increase their shelf life and stability and facilitate their distribution before consumption (Deák 2014). The traditional technology used for beverage preservation is thermal pasteurization, which can be generally classified into low-temperature/long-time (LTLT) and high-temperature/short-time (HTST) processes, whose application depends on the food substrate to

be processed. Usually, LTLT is adopted for milk and dairy products (namely 63°C for at least 30 min) (Roberts *et al.* 2005), while HTST pasteurization is suitable for fruit juices (Chen *et al.* 2013), where temperatures from 72 to 108°C with holding times (HTs) of at least 15 s are employed. The aim of thermal pasteurization is to kill pathogens and substantially reduce the number of spoilage microorganisms through a suitable time/temperature combination. The major drawback of both methods is represented by the degradation of taste, color, flavor and nutritional quality of foods (Charles-Rodríguez *et al.* 2007). Moreover, the beverages that have to be bottled after thermal treatments also require expensive aseptic filling systems to prevent post-processing contamination risk. For these reasons, during the previous years, various novel pasteurization technologies have been developed, including thermal and non-thermal methods (Ramaswamy *et al.* 2004). Novel techniques employing lower temperatures should be especially appreciated as they could maintain the "fresh-like" characteristics and the high nutritional values in treated foodstuff, also allowing both container and content to be submitted to the thermal stabilization treatment at the same time, saving time and resources (Guillard *et al.* 2010).

Recently, a new thermal treatment to inactivate microorganisms in beverages has been patented (Koulik and Zavadtsev 2010); the basic idea is a radiofrequency (RF) heating process with temperature rates higher than 28°C/s and final temperatures up to 65°C, in combination with a pulsed electric field. According to its authors, the lethal effect on microorganisms should be due to the irreversible formation of pores in the lipid cell coatings, thanks to the rapid temperature increase. Besides this patent, however, there is no record of microorganisms inactivation by means of such a heating process in the scientific literature, nor the effect of rapid temperature increases has ever been investigated as a possible mechanism for microorganisms inactivation.

On the basis of these considerations, the authors decided to test the microorganisms inactivation effectiveness of thermal treatments characterized by very rapid temperature increases and relatively low final temperatures; differently from the Koulik and Zavadtsev patent, however, the authors focused on the temperature role, thus leaving aside any electric field contribution. Hence, the process tested by the authors aims to cause a lethal effect on microorganism by means of a pure heat transfer process. The experimental evaluation of the effectiveness of the new thermal treatment has been carried out on aqueous contaminated suspensions according to Juice HACCP (Hazard Analysis and Critical Control Points) Regulation of US Food and Drug Administration (FDA U.S. Food and Drug Administration 2001), following the UNI EN test procedures for bacteria (UNI EN 2009) and yeast (UNI EN 2008) meticulously.

MATERIALS AND METHODS

Thermal Treatment System

To obtain the rapid temperature increases required by the new thermal treatment, a properly designed test bench, based on the ohmic dissipation effect, has been performed. Each suspension has been pumped inside a stainless steel tube which was subjected to a voltage drop across its terminals. In this way, the inner tube surface, heated by the ohmic dissipation, transfers heat to the flowing suspension, whose temperature increment (ΔT) can be related to the electric power dissipation (W_{el}) through the following relation

$$\eta W_{el} = \eta i^2 R = c_p G \Delta T \tag{1}$$

where i represents the electric current flowing in the metallic tube, R is the tube electrical resistance, G is the suspension mass flow, c_p is the suspension specific heat at constant pressure and η represents the heat transfer efficiency of the system, which, in the tests carried out, was found to be approximately 0.75.

Considering the suspension as an incompressible fluid, both its density δ and volumetric flow Q can be considered constant along the whole tube:

$$Q = \frac{G}{\delta} = \text{constant} \tag{2}$$

Given also the constant tube diameter, the flow section area (A) does not change along the tube; it results then to

$$\frac{Q}{A} = \frac{l}{\Delta\tau} = v \tag{3}$$

where $\Delta\tau$ represents the thermal treatment duration, i.e., the time required for the suspension to flow through the whole tube length (l) and v is the mean flow velocity.

From Eqs. (2) and (3), it follows that

$$G = \frac{lA\delta}{\Delta\tau} \tag{4}$$

and Eq. (1) becomes

$$\eta i^2 R = c_p \frac{lA\delta}{\Delta\tau}\Delta T \tag{5}$$

Hence, the rate of temperature rise (ϑ) of the suspension can be obtained as

$$\vartheta = \frac{\Delta T}{\Delta\tau} = \frac{\eta i^2 R}{c_p lA\delta} \tag{6}$$

where the metallic tube resistance R is related to the metal resistivity ρ, to the tube length l and to the tube annular section S according to the relation

$$R = \frac{\rho l}{S} \tag{7}$$

It follows that, given a certain rate of temperature rise (ϑ), the necessary electrical current i can be obtained as

$$i = \sqrt{\frac{\vartheta c_p \delta A S}{\eta \rho}} \tag{8}$$

This implies that, once the tube diameter and thickness have been established, the electrical current is immediately determined for each desired rate ϑ.

The additional fundamental parameter of the thermal treatment process, i.e., the temperature increment (ΔT) can be obtained by Eqs. (3) and (6):

$$\Delta T = \vartheta \Delta \tau = \frac{\vartheta l A}{Q} \tag{9}$$

Hence, the process parameters ϑ and ΔT may be easily controlled by means of the electric current i and the volumetric flow Q.

A schematic representation of the thermal treatment system is reported in Fig. 1. A Watson-Marlow peristaltic pump 120S/DV (Watson-Marlow Inc., Wilmington, MA, USA), endowed of a Marprene tube, has been used to pump the suspension into the stainless steel tube (AISI 316, outer diameter 1.59 mm and thickness 0.36 mm – respectively 1/16 in. and 0.014 in. - Swagelok Company, Soloh, OH, USA), while a laboratory grade DC (Direct Current) power supply system has been used to generate the needed electrical current. The use of the peristaltic pump was preferred both for an accurate mass flow control (±1% accuracy) and to better preserve the suspension from contamination.

The fluid temperature has been measured both at the inlet (T_1, initial temperature) and at the outlet (T_2, final temperature) of the heated tube by means of K-type thermocouples connected to a USB TC-08 Pico Tech Data Logger (Pico Technology, Cambridgeshire, PE, UK). Heat transfer to ambient has been minimized by the use of a proper insulating tape applied to the whole process line. Table 1 resumes the operative parameters employed for the tests specified in the "Test procedure and microbial count" subsection.

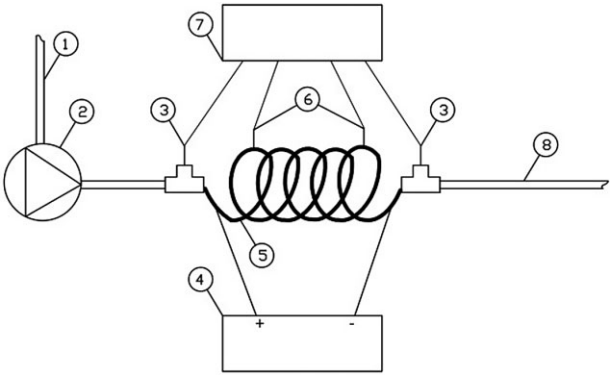

FIG. 1. SCHEMATIC REPRESENTATION OF THE TEST BENCH
1, From feed; 2, Peristaltic pump; 3, K-type immersion thermocouple; 4, Power supply; 5, Metallic tube; 6, K-type thermocouple; 7, Data logger; 8, To drain.

TABLE 1. OPERATIVE PARAMETERS OF THE THERMAL TREATMENT SYSTEM

l (m)	A (mm²)	ϑ (°C/s)	i (A)	T_1 (°C)	T_2 (°C)	Q (mL/min)
1.5	0.6	20	11.5	30	60	36
					65	31
		30	13.3		60	54
					65	46

ϑ, rate of temperature rise; A, flow section area; i, electric current; l, tube length; Q, volumetric flow; T_1, initial temperature; T_2, final temperature.

Microorganisms

The strains used in this study were the bacteria *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Listeria innocua* (ATCC 33090) and the yeast *Candida albicans* (ATCC 76615). These species were selected on the basis of their importance in food processing, as they are common food- and waterborne pathogens and are defined by the World Health Organization as organisms whose detection is an index of pollution/contamination (WHO 2011). In particular, *P. aeruginosa* inactivation is deemed very important in the field of the beverage industry because it is a common environmental organism which can multiply in water environments and on the surface of suitable organic materials in contact with water, although there is no evidence that normal supplied drinking water is a source of *P. aeruginosa* infection in the general population. However, the growth of these organisms as biofilm in the pipelines at the bottling plant can cause the presence of high numbers of *P. aeruginosa* in packaged water and drinks which can be associated with complaints about taste, odor and turbidity (WHO 2011). *E. coli* is present in large numbers in the normal intestinal flora of humans and animals. Therefore, *E. coli* is a suitable index of fecal contamination and is used as indicator organism for water quality monitoring (Spinks *et al.* 2006). The enteropathogenic *E. coli* strains are among the most commonly detected contaminants in foodborne outbreaks, where drinking water has been involved as a vehicle (WHO 2011; Caprioli *et al.* 2014). *Staphylococcus* species are ubiquitous in the environment and they can easily contaminate a variety of foods. Because staphylococci are present in the nose and throats and on the hair and skin of many healthy individuals, food handlers are frequently the source of food contamination in staphylococcal outbreaks (Todd 2014). *Listeria* species

increasingly emerge as significant foodborne pathogens. As they are widely distributed in nature, the human contact occurs frequently although infections are relatively rare. Moreover, they can survive refrigeration temperature and grow in several environments, including water (Magalhães *et al.* 2014). The pathogenic yeast *C. albicans* is a normal inhabitant of the intestine of humans and animals and is also present on the skin of healthy individuals (Calderone 2002). Therefore, it can be considered both an indicator of fecal contamination or incorrect product handling. Moreover, *C. albicans* can be regarded as opportunistic species, causing spoilage as a consequence of mistakes in manufacturing or storage (Stratford 2006).

Test Suspension

All the microorganisms employed in the tests were properly cultured on trypticasein soy agar (Laboratorios Conda S.A., Madrid, Spain), with the exception of *L. innocua* and *C. albicans*, propagated respectively on Listeria selective agar, Oxford Formulation (Oxoid Ltd, Hampshire, UK) and on malt extract agar (Laboratorios Conda S.A.).

According to the adopted UNI EN standards, an initial suspension was prepared in buffered peptone water from the fresh culture of each microorganism: as prescribed by the standards, for the yeast *C. albicans*, a concentration of 10^7 cfu/mL of initial suspension was adopted, while for all the bacteria, the initial concentration was 10^8 cfu/mL.

The test suspensions were prepared starting from the initial suspensions, by adding hard water (19.84 g/L $MgCl_2$, 46.24 g/L $CaCl_2$, 35.02 g/L $NaHCO_3$ in sterile distilled water) and bovine albumin solution or sucrose solution (0.3 g/L and 10 g/L, respectively) as interfering substance, thus creating a substrate characterized by ion content and organic matter similar to beverages such as aromatized water, fruit juices, etc. The volumetric composition of each test suspension realized was 10% of initial suspension, 80% of hard water and 10% of interfering substance.

Test Procedure and Microbial Count

The test suspensions have been submitted to a protocol panel classified by the three main process parameters: the rate of temperature rise (ϑ, 20 or 30°C/s), the final process temperature T_2 (60 or 65°C) and the holding time (HT) at the temperature T_2 (0 or 60 s). For fast referencing purpose, each protocol has been labeled using the values of the above-mentioned three parameters (i.e., ϑ-T_2-HT): each of the resulting eight tested protocol variants, which are presented in Table 2, has been repeated using both the two interfering substances (bovine albumin and sucrose).

For each combination of protocol variant, microorganism and interfering substance, four replicate tests have been carried out. After each single treatment, the test bench has been completely sanitized using 0.5% sodium hypochlorite and then rinsed with sterile distilled water.

TABLE 2. TESTED PROTOCOL VARIANTS

ϑ (°C/s)	T_2 (°C)	HT (s)	Variant code
20	60	0	20-60-0
		60	20-60-60
	65	0	20-65-0
		60	20-65-60
30	60	0	30-60-0
		60	30-60-60
	65	0	30-65-0
		60	30-65-60

ϑ, rate of temperature rise; HT, holding time; T_2, final temperature.

Before the thermal treatment, each test suspension has been preheated in a thermostatic bath for 30 min at 30°C and at the end of the thermal treatment, the test suspension has been stored in an ambient at 4°C for 1–2 h. After this storage, a 1 mL sample of test suspension has been divided into three aliquots and spread onto three separate agar plates, which have been subjected to 48 h of incubation: this has been carried out at 37°C for the bacteria and at 30°C for the yeast. Once the incubation period has been terminated, the colonies on each agar plate have been counted to determine the microbial concentration (number of cells per milliliter of test suspension) as sum of the three values. In order to prevent any measurement or procedure error, the whole plating and counting processes have been repeated twice for each test solution and the final microbial concentration N_f was evaluated as the average of the two samples.

The initial microbial concentration of each test suspension has been determined by serial decimal dilutions: a sample of test suspension not subjected to thermal process has been diluted in sterile peptone water and plated. As prescribed by the standards, the 10^{-6} and 10^{-7} dilutions were adopted for bacteria, while 10^{-5} and 10^{-6} dilutions were used for the yeast.

For each of the two dilutions, the number N of cells per milliliter of test suspension was calculated as follows

$$N = \frac{c}{n \cdot 10^{DF}} \tag{10}$$

where c is the number of cfu counted in the plates considered, n is the volume of the sample seeded in the plates (here expressed in milliliter) and DF is the exponent of the dilution factor (i.e., −5, −6 or −7). The total number N_i of cells per milliliter of initial suspension has been then obtained as the average value between the two dilutions.

The reduction of microbial concentration in the suspension submitted to the thermal treatment has been evaluated, according to the previously mentioned UNI EN standards, as

$$R = N_i/N_f \quad (11)$$

where N_i and N_f represent the initial and the final microbial concentration.

The microorganism inactivation has been considered effective when the reduction of counts gave as result Log$R \geq 5$ for the bacteria and Log$R \geq 4$ for the yeast.

According to the above-mentioned UNI EN standards for microbial count on agar plates, the final concentration can be considered measurable if it is within the limits prescribed, which are 14 cfu in the whole solution sample for the lower limit and 330 cfu on a single plate for the upper limit; outside of these limits, the count result was indicative of the full effectiveness (<14) or ineffectiveness (>330) of the thermal test performed.

Validation Procedures and Controls

Some validation procedures and controls were also performed for each strain and interfering substance combination:

(1) 2 mL of the test suspension containing each of the two interfering substances were plated in the proper culture media and incubated, with the aim to check the absence of any lethal effect of the interfering substances on the selected strains under the test conditions;

(2) 2 mL of rinsing water were collected and plated in the proper culture media and incubated, to control the absence of growth, thus ascertaining the disinfection effectiveness of the test bench;

(3) rinsing water was submitted to the dilution-neutralization test for the evaluation of the bactericidal activity of the chemical disinfectants to verify the absence of residual toxic effect after the test bench disinfection. For this purpose, a 0.5% sodium hypochlorite solution was used as positive control.

RESULTS AND DISCUSSION

The results of the tests carried out are presented in Table 3 and Table 4. Here, the ratio between the number of effective tests and the number of total tests is reported for each protocol variant and for the two interfering substances.

As can be observed by the data reported in Table 3, the protocols with $T_2 = 65$°C proved to be effective against all the microorganisms tested, with both interfering substances. In particular, the following residual microbial loads and Log$R$ resulted for each strain tested: *P. aeruginosa*, residual load ≤250 cfu/mL and Log$R \geq 5.1$; *S. aureus*, residual load ≤190 cfu/mL and Log$R \geq 5.2$; *E. coli*, residual load ≤41 cfu/mL and Log$R \geq 6.1$; *L. innocua*, residual load ≤170 cfu/mL and Log$R \geq 5.1$; *C. albicans* residual load <14 cfu/mL and Log$R > 5.1$.

On the contrary, the protocols with $T_2 = 60$°C (Table 4) proved to be generally ineffective, except for *C. albicans* which was susceptible to all the protocols (residual load <14 cfu/mL and Log$R > 5.1$). For the other strains, the $T_2 = 60$°C tests became partially (*E. coli*) or totally (*P. aeruginosa*) effective only with $HT = 60$ s. More in detail, with $T_2 = 60$°C, the residual load of *S. aureus* was always

TABLE 3. EFFECTIVE TESTS OVER TOTAL TESTS FOR PROTOCOLS WITH FINAL TEMPERATURE $T_2 = 65$°C

	20-65-0		20-65-60		30-65-0		30-65-60	
Strain	Albumin	Sucrose	Albumin	Sucrose	Albumin	Sucrose	Albumin	Sucrose
Pseudomonas aeruginosa	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Escherichia coli	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Staphylococcus aureus	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Listeria innocua	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Candida albicans	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

TABLE 4. EFFECTIVE TESTS OVER TOTAL TESTS FOR PROTOCOLS WITH FINAL TEMPERATURE $T_2 = 60$°C

	20-60-0		20-60-60		30-60-0		30-60-60	
Strain	Albumin	Sucrose	Albumin	Sucrose	Albumin	Sucrose	Albumin	Sucrose
Pseudomonas aeruginosa	0/4	0/4	2/4	4/4	0/4	0/4	4/4	3/4
Escherichia coli	0/4	0/4	1/4	1/4	0/4	0/4	1/4	0/4
Staphylococcus aureus	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Listeria innocua	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Candida albicans	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

>990 cfu/mL with a Log$R$ < 4.7. Similar results were observed for *L. innocua*, with a residual load constantly >990 cfu/mL and a Log$R$ < 4.4. Regarding *E. coli*, only one out of four protocols 20-60-60 with each of the two interfering substances used (albumin: residual load 310 cfu/mL, Log$R$ 5.3; sucrose: residual load 110 cfu/mL, Log$R$ 5.4) and one out of four protocols 30-60-60 performed with albumin as interfering substance (330 cfu/mL, Log$R$ 5.2) resulted effective. In the other T_2 = 60°C tests, *E. coli* Log$R$ was always ≤4.9, with a minimum residual bacterial load of 480 cfu/mL, but counts were often >990 cfu/mL. *P. aeruginosa* was generally susceptible to those treatments associated to HT = 60 s (protocol 20-60-60 and 30-60-60), showing a residual load ≤250 cfu/mL and Log$R$ ≥ 5.1, except for two out of four tests in the presence of albumin, where the residual load was ≥650 cfu/mL and Log$R$ ≤ 4.6. Similar to what was observed for the other strains, treatments 20-60-0 and 30-60-0 were ineffective on *P. aeruginosa* (residual bacterial load ≥520 cfu/mL, Log$R$ ≤ 4.8).

The data reported in this study seem to indicate a threshold value in the final temperature of the rapid temperature increase that determines the success or not of the thermal treatment. Indeed, for all the tested strains, consistent count reductions were obtained when T_2 was 65°C, while counts remained generally high when T_2 was 60°C. However, in some particular cases (i.e., *P. aeruginosa* and *E. coli*), an HT of 60 s allowed to boost the effectiveness of the thermal treatment tested with a final temperature T_2 set at 60°C.

The almost equal results found employing rates of temperature rise of both 30°C/s and 20°C/s suggest a possible further reduction of this parameter. This reduction could allow substantial advantages in terms of technical requirements and lower power consumption, especially when large-scale applications are considered. Additional researches are hence planned to assess the effectiveness and limits of the rate of temperature rise, maintaining the final temperature at 65°C.

CONCLUSION

As overall outcome, the new thermal process proved to effectively reduce the microbial load of all the tested strains, with both interfering substances, of at least 5 Log units when the T_2 was 65°C, apart from the rate of temperature rise (ϑ) and without requiring an HT.

The findings of this study suggest that the process could be conveniently applied in the beverage industry technology.

The use of a final temperature not exceeding 65°C would allow to better preserve the sensorial and nutritional features of the beverage compared with higher temperatures treatments (Qin *et al.* 1995); moreover, a low final temperature is in agreement with energy-saving issues.

Thanks to the short duration of the treatment and to the relatively low maximum temperature (65°C), which does not damage the plastic materials commonly used in the beverage industry for bottles packaging, the treatment is compatible with already packaged products. Its future use online with the filling process could then be hypothesized. Nowadays, most liquid foods, such as beverages, milk, fruit and vegetables juices, are pasteurized by HTST method and then bottled or packaged under rigidly maintained sterile conditions (Deák 2014). The use of a technology based on this treatment on already bottled products would hence avoid the very expensive filling system in an aseptic environment and the risk of the post-processing contamination. However, the rapid heating of packaged foods is not currently achievable by the traditional convection or conduction heat transfer, while the use of RF generators could instead allow a rapid and uniform heating of the product (Rowley 2001). This novel application has emerged and has attracted a considerable interest in recent years (Marra *et al.* 2009; Lombardo *et al.* 2014). The authors of this work are currently involved in the design of a proper laboratory device to test and assess the effectiveness of thermal processes performed by means of RF heating.

ACKNOWLEDGMENTS

This research is part of the Dynamic Irreversible Thermoporation (DIT) project supported by PO FESR 2007/2013 fund of European Union.

REFERENCES

CALDERONE, R.A. 2002. *Candida and Candidiasis*, p. 4, ASM Press, Washington, DC.

CAPRIOLI, A., MORABITO, S. and SCAVIA, G. 2014. Bacteria: Shiga toxin-producing *Escherichia coli* and other pathogenic *Escherichia coli*. In *Encyclopedia of Food Safety – Volume 1: "History, Science and Methods"* (Elsevier Inc, ed.) pp. 417–423, Elsevier, San Diego, CA.

CHARLES-RODRÍGUEZ, A.V., NEVÁREZ-MOORILLÓN, G.V., ZHANG, Q.H. and ORTEGA-RIVAS, E. 2007. Comparison of thermal processing and pulsed electric fields treatment in pasteurization of apple juice. Food Bioprod. Process *85*, 93–97.

CHEN, Y., YU, L.J. and RUPASINGHE, V. 2013. Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: A mini-review. J. Sci. Food Agric. *93*, 981–986.

DEÁK, T. 2014. Pasteurization. In *Encyclopedia of Food Safety – Volume 3: "Foods, Materials, Technologies and Risks"* (Elsevier Inc, ed.) pp. 219–224, Elsevier, San Diego, CA.

FDA U.S. FOOD AND DRUG ADMINISTRATION. 2001. *Final Rule: Hazard Analysis and Critical Control Point (HACCP);*

Procedures for the safe and sanitary processing and importing of juice. 01–1291, 66, 13, 6138–6202.

GUILLARD, V., MAURICIO-IGLESIAS, M. and GONTARD, N. 2010. Effect of novel food processing methods on packaging: Structure, composition, and migration properties. Crit. Rev. Food Sci. Nutr. *50*, 969–988.

KOULIK, P.P. and ZAVADTSEV, A., 2010. *Methods and device for sterilising a liquid*, United States Patent Application Publication No. US2010/0297313 A1.

LOMBARDO, R., RUBINO, T. and CAMMALLERI, M. 2014. Dielectric properties of fruit nectars at low frequencies in the RF spectrum. Int J. Food Prop.

MAGALHÃES, R., MENA, C., FERREIRA, V., SILVA, J., ALMEIDA, G., GIBBS, P. and TEIXEIRA, P. 2014. Listeria monocytogenes. In *Encyclopedia of Food Safety – Volume 1: "History, Science and Methods"* (Elsevier Inc, ed.) pp. 450–461, Elsevier, San Diego, CA.

MARRA, F., ZHANG, L. and LYNG, J.G. 2009. Radio frequency treatment of foods: Review of recent advances. J. Food Eng. *91*, 497–508.

QIN, B.L., POTHAKAMURY, U.R., VEGA, H., MARTIN, O., BARBOSA-CANOVAS, G.V. and SWANSON, B.G. 1995. Food pasteurization using high-intensity pulsed electric fields. Food Technol. (Chicago) *49*, 55–60.

RAMASWAMY, H.S., CHEN, C. and MARCOTTE, M. 2004. Novel processing technologies for food preservation. In *Processing Fruits: Science and Technology* (D.M. Barrett, ed.) pp. 201–217, CRC Press, Boca Raton, FL.

ROBERTS, T.A., CORDIER, J.L., GRAM, L., TOMPKIN, R.B., PITT, J.I., GORRIS, L.G.M. and SWANSON, K.M.J. 2005. Milk and dairy products. In *Microorganisms in Foods 6* (Kluwer Academic/Plenum Publishers, New York, ed.) pp. 643–715, Springer, New York, NY.

ROWLEY, A.T. 2001. Radio frequency heating. In *Thermal Technologies in Food Processing* (P. Richardson, ed.) pp. 163–177, CRC Press, Boca Raton, FL.

SPINKS, A.T., DUNSTAN, R.H., HARRISON, T., COOMBES, P. and KUCZER, G. 2006. Thermal inactivation of water-borne pathogenic and indicator bacteria at sub-boiling temperatures. Water Res. *40*, 1326–1332.

STRATFORD, M. 2006. Food and beverage spoilage yeasts. In *The Yeast Handbook Volume 2* (Amparo Queral, Graham Fleet, ed.) pp. 364–366, Springer-Verlag, Berlin; Heidelberg.

TODD, E.C.D. 2014. Staphylococcus aureus. In *Encyclopedia of Food Safety – Volume 1: "History, Science and Methods"* (Elsevier Inc, ed.) pp. 530–534, Elsevier, San Diego, CA.

UNI EN. 2008. *UNI EN 1650:2008. Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test methods and requirements (phase 2, step 1)*. ed UNI – Ente Nazionale Italiano di Unificazione.

UNI EN. 2009. *UNI EN 1276:2009. Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas. Test methods and requirements (phase 2, step 1)*. ed UNI – Ente Nazionale Italiano di Unificazione.

WHO. 2011. *Guidelines for Drinking-Water Quality – Volume 1. Recommendations*, World Health Organization Press, Geneva, Switzerland.