

Impact of Novel Nonthermal Processing on Food Quality: Sustainability, Modelling, and Negative Aspects

Lead Guest Editor: Anet Režek Jambrak

Guest Editors: Tomislava Vukušić, Larysa Paniwnyk, Ilija Djekić,
and Francesco Donsi





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Editorial

Impact of Novel Nonthermal Processing on Food Quality: Sustainability, Modelling, and Negative Aspects

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Novel food processing technologies, including nonthermal processing techniques, such as electro-technologies (pulsed light, pulsed electric field, cold plasma, e-beam processing, etc.), mechanical processing (ultrasound and hydrodynamic cavitation), pressure-based technologies (high-pressure processing, high-pressure homogenization, supercritical fluid extraction, subcritical water extraction, etc.), and novel thermal processing techniques, such as ohmic heating and dielectric heating (radio frequency and microwave heating), have attracted increasing attention in the recent years. The implementation of novel nonthermal and thermal technologies in food processes is driven by the growing demand for fresh-like, more natural food products, which, however, ensure the convenience of large-scale commercial distribution and long shelf life. Therefore, food researchers are currently addressing the exploration and development of alternatives to conventional food processing technologies, not only to improve food preservation but also to product quality and sustainability, without jeopardizing food safety.

Food and Drug Administration (FDA) was among the first regulatory agencies to request from the Institute of Food Technologists (IFT) to report on the effectiveness of microbial inactivation by alternative food processing technologies. The idea was to develop applications of novel nonthermal technologies, eventually in combination with advanced thermal technologies, to ensure food safety by satisfying the requirement of a minimum 5-log reduction of the endogenous flora. In support to this trend, the latest UN

Sustainable Development Goals also promote the development of sustainable technologies.

The researchers in food processing have to pursue food safety, bearing in mind food security and the reduction of the impact on the environment. The aim of processing by using alternative food processing technologies is to achieve the desired inactivation of microorganisms (food safety), and concurrently to reduce energy consumption, optimize time-consuming processes, and satisfy the consumers' requests. Nowadays, one of the biggest challenges is to scale up the readiness level of these novel technologies to an industrial level.

Thermal techniques have been used for decades, with the results in terms of inactivation of microorganisms, which are not debatable; however, high-temperature processing is often responsible for the deterioration of nutritive, functional, and organoleptic properties. Therefore, several nonthermal techniques had been evaluated for their potential in food preservation. So far, only high-pressure processing satisfied the requirements in terms of microbial inactivation, when used alone in food preservation, whilst the use of other nonthermal processing techniques is industrially viable only in combination with moderate heating, to ensure the required food preservation effect. In addition, the mechanisms underlying the inactivation of microorganism by novel technologies have not been fully elucidated and are still under study, with several proposed action plans still ongoing for each technique. Finally, more

reliable shelf life studies on nonthermally processed products are necessary, because of the risks associated with sublethal injuries, which lead to the revitalization and possible “stress” effect on microorganisms, where they are not killed but are “under stress.” This phenomenon can be detected and is called viable but nonculturable state. Such microorganisms can revitalize and organize to set a biofilm formation, considered as a very strong network formed of microorganisms’ cell and carbohydrates, whose formation is very hard to break.

These techniques have been extensively investigated in terms of their impact on food quality, the nutritional value of food, microbiological safety, drying, extraction, enzyme inactivation rate, and sensory properties and other advantages on product technological properties or functionality. However, there are still major unresearched gaps, especially concerning the evaluation of negative aspects of application of novel nonthermal processing on food quality, stability of food during shelf life upon nonthermal processing, negative sensory properties of food treated by novel nonthermal and thermal techniques, life cycle assessment and sustainability of novel nonthermal and thermal processing techniques, advantages of novel nonthermal and thermal processing in terms of energy consumption and quality, usage of green techniques, “green” solvents, “green” extraction processes, and impact on food quality. The scope for this special issue is to promote articles, which describe the current state of the art in the mentioned areas and to attract and “especially welcome” research papers dealing with proposed unresearched topics.

This special issue provides an overview of the focused usage of novel technologies for assuring food safety, quality, and low impact on the environment. In particular, it aims at emphasizing the usage of alternative nonthermal processing as sustainable processing technologies. In addition, this special issue also tries to address the optimization and combination of advanced processing technologies by promoting advantages of each nonthermal and advanced thermal ones, by avoiding negative factors and negative influence on food products. The special issue also emphasizes the negative effects of application of novel technologies in terms of radical formation. Production of free radicals is a negative side effect in using specific technologies (such as ozonization, radiation, ultrasound processing, plasma processing, and advanced oxidative processes), so it is necessary to optimize processing conditions in order to reduce processing time, and possibility for free radical formation, thereby reducing negative effect on products.

Special focus is given to determine the required lethality effect on microorganisms (without the possibility for revitalization) and induce biofilm deterioration and/or prevention. It also focuses on ensuring the safety of packaging materials of products that were treated by nonthermal processing, as well as efficient surface decontamination techniques, to ensure microbial inactivation and avoid contamination of container or packaging. Finally, this issue also treats the quality aspects of novel food processing technologies, especially for what concerns the observation and control of final product quality using various analytical

methods, such as the analysis of chemical composition, and of physical and sensory properties of food, in terms of color, texture, taste, odor, flavor, etc.

Two future challenges in developing novel food processing technologies, which clearly emerged from the articles collected in this issue, are (i) the development of a compact tool for evaluating hygienic design of novel nonthermal or thermal food-processing equipment, when scaled up to industrial level, due to the redundancy of the several requirements outlined in different standards and regulations related to hygienic design and (ii) the improvement of the overall environmental performance of these novel technologies, compared to conventional technologies, with improved nutritional and quality characteristics.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

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Research Article

Quality Changes of Orange Juice after DPCD Treatment

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Dense phase carbon dioxide (DPCD) offers advantages of enhanced physical and nutritional qualities during the processing of juices. Here, freshly squeezed orange juice was treated with DPCD, and changes of physical properties and volatile components were investigated and compared with the original untreated and thermally treated samples. The correlations among physicochemical properties were also examined based on Pearson correlation, cluster analysis (CA), and principal component analysis (PCA). Significant correlations were found among the particle size, color parameters, and volatile compounds in the DPCD-treated samples. The 12 parameters were clustered into three groups using CA and PCA, and the eight volatile compounds were separated within the three groups. Nonanal and citronellol were clustered in group I, and they increased for a longer duration of more than 40 min with higher levels than the control. Parameters in group II included $D(4,3)$, L^* , a^* , ethyl butyrate, and *trans*-2-hexenol, and they linearly decreased after 10–60 min DPCD treatment. The parameters of b^* and monoterpenes were clustered in group III, and they decreased within 40 min of DPCD treatment and then increased to an intermediate level. In addition, PCA clearly showed that the orange juice samples under DPCD for 10–60 min formed a “U” shape on the two-dimensional plot and that the samples treated by DPCD for 10 min and 20 min were closer to freshly squeezed orange juice than the heat-treated orange juice. This indicated that the nonthermal DPCD process offers the potential to be used more extensively in juice products.

1. Introduction

As a nonthermal process, the use of dense phase carbon dioxide (DPCD) is attractive because it causes less degradation to the quality of juices and beverages than thermal processes. The effects of DPCD on the nutrient content, stability, color, and sensory quality of foods have been widely studied. Many results have shown that DPCD does not modify the chemical, physical, and organoleptic qualities such as pH, °Brix, and turbidity of fresh liquid foods [1–3]. However, most results on the volatile components have shown negative conclusions: in most cases, the content of volatile components was reduced significantly. Gasperi et al. [4] reported that the contents of esters and aldehydes in fresh apple juice were reduced by more than half after DPCD treatment. Plaza et al. [5] reported that DPCD treatment reduced the total MS ion chromatogram peak area by 35% in

guava purée. It is possible that the volatile components were removed by CO₂ during depressurization. However, a few reports showed no significant change [6] or even an increase of some volatile components [7] after DPCD treatment. It is well known that the cloud juice is a liquid-solid system consisting of the serum and a heterogeneous water-insoluble phase consisting of small particles [8, 9]. In orange juice, there is a clear partitioning of the volatile components between the insoluble pulp and the aqueous serum [10]. Moreover, when juice is immersed in dense CO₂, the particle size, solubility of volatile substances, and the distribution of pigments could change under certain pressure and temperature conditions [11–13]. For example, in carrot juice [14] and peach juice [15], the particle size increased significantly. In contrast, the b^* value (yellowness/blueness) decreased in grapefruit juice [1] and mandarin juice [16] after DPCD treatment.

To date, little information is available on the relationship between changes in different properties under DPCD treatment, and determination of the quality changes with prolonged DPCD duration remains confusing. The present study focuses on the changes of particle size, color, and volatile components of freshly squeezed orange juice after DPCD treatment. Cluster analysis (CA) and principal component analysis (PCA) were used to investigate the correlations of the parameters after DPCD treatment; furthermore, the quality of the juices after DPCD treatment was compared with the original freshly squeezed and the heat-treated juice.

2. Materials and Methods

2.1. Orange Juice Preparation. Fresh Hamlin oranges were peeled manually and then mashed using a domestic blender. The pulp was then packed into a nylon cloth (80 mesh) and squeezed to obtain the juice. Two liters of juice were collected and mixed well. The juice was then divided into three groups. One was the untreated fresh samples, while the other two were immediately subjected to DPCD treatment and thermal treatment (90°C, 60 s), respectively. After treatment, all of the samples were stored at 4°C and all parameters were determined within 24 hours.

2.2. DPCD Processing. The DPCD system in this study was the same as that previously described by Zhou et al. [14]. A high-pressure vessel was heated to 55°C and then evacuated. Under vacuum conditions, 200 mL juice was drawn into the pressure vessel by opening the sample inlet valve for each treatment, and then the valve was closed immediately. Meanwhile, the CO₂ inlet valve was opened, and the pressure was increased to 40 MPa at the rate of 10 MPa/min. The high pressure was applied for 10, 20, 30, 40, 50, and 60 min, and six DPCD-treated samples were thus collected.

2.3. Particle Size and Color Analysis. The particle size distribution was determined using an LS 230 laser diffraction particle analyzer (Beckman Coulter Inc., Brea, CA, USA) as described by Zhou et al. [15]. The volume mean diameter $D(4, 3)$ was determined.

The color parameters (L^* , a^* , b^*) were measured using an automatic colorimeter (WSC-S, Shanghai Jingmi Instrument Co., Shanghai, China). The instrument was calibrated using a black cuvette for attenuated reflection and a white ceramic tile as the reference standard (National Standard Research Center, Beijing, China).

2.4. Determination of Volatile Compounds. The volatile components were extracted using a solid phase micro-extraction (SPME) system. An 8 mL juice sample was placed in a 15 mL vial containing a stirring bar and 2.56 g NaCl, and then the vial was placed into a water bath at 40°C and stirred at 200 rpm. After allowing 10 min for temperature equilibration, a 100 μm polydimethylsiloxane-coated fiber (Supelco Ltd., Bellefonte, PA, USA) was inserted and allowed to extract the volatile components for 20 min.

The volatile compounds were separated and detected using a 7890A/5975C MS system equipped with an HP-5 column (30 m × 0.25 mm × 0.25 μm thick film) (Agilent Technologies Inc., Santa Clara, CA, USA). The GC injection port was set at 220°C, and the MS detector was operated in the scan mode with 70 eV electron impact, scanning throughout the 30–450 m/z range. The multiplier was set at 1.0 kV, and the source temperature and transfer lines were maintained at 230 and 280°C, respectively. The oven program was as follows: initial 50°C for 2 min, increased by 4°C/min to 160°C, increased by 10°C/min to 220°C, and then maintained for 3 min at 220°C.

The C₈-C₂₄ standard alkane and standard flavor compounds of ethyl butyrate, *trans*-2-hexenol, α -pinene, phellandrene, limonene, linalool, nonanal, and citronellol (Sigma-Aldrich, Shanghai, China) were injected under the same chromatographic conditions. The volatile flavor compounds were identified according to the NIST 2013 database for standard compounds, and then the retention indices were calculated from the alkane series.

2.5. Statistical Analysis. Each sample was tested in triplicate, and the data were expressed as the mean ± standard deviation. All data were expressed as percentage change relative to the corresponding control with the average of the control sample as 100%. ANOVA, multiple correlation, and PCA were performed using JMP Pro 10 software (SAS Institute Inc., Cary, NC, USA). CA was performed and visualized using MultiExperiment Viewer software (<http://www.tm4.org/#/welcome>).

3. Results

3.1. Changes of Particle and Color Parameters. The effects of DPCD treatment on the physical parameters of orange juice are shown in Figure 1. The particle size of samples treated by DPCD for 10 min was not significantly different ($p > 0.05$) from the untreated (control) sample but was reduced significantly—approximately 20%—by the 20 min treatment. The changes in particle size of the orange juice remained insignificant until the DPCD treatment time reached 50 or 60 min. In other words, the $D(4, 3)$ values of samples treated for 30 or 40 min were not significantly different from those of the sample treated for 20 min. In addition, there were no statistically significant differences in particle size between control and thermally treated samples (99.34% that of the fresh sample), and the smaller values of DPCD processing correspondingly resulted (71.83–81.13%).

No significant differences were found ($p > 0.05$) among the L^* values of all the treated and control samples, while a^* values of all the DPCD-treated samples were lower than those of the control sample ($p < 0.05$). The b^* value reached the lowest magnitude after 30 and 40 min of DPCD treatment and then slightly increased (Figure 1). All of the b^* values were higher than those of the control sample, while b^* value of the heat-treated sample was close to ($p > 0.05$) the lowest value of DPCD-treated samples, which occurred after DPCD processing for 20 and 30 min.

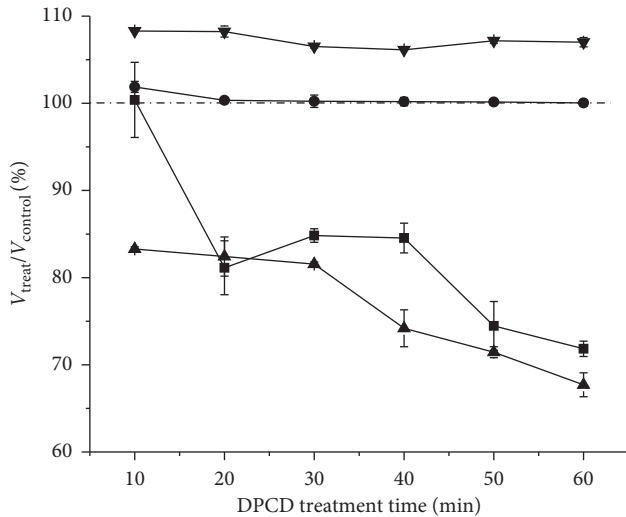


FIGURE 1: Effect of DPCD processing (40 MPa, 55°C) on particle size and color in orange juices: ■, $D(4,3)$; ●, L^* ; ▲, a^* ; ▼, b^* . Each value represents mean \pm standard deviation of three replicates. V in the y -axis indicates the determined value of $D(4,3)$, L^* , a^* , and b^* , taking the average value of the untreated sample as control.

3.2. Changes of Volatile Compounds. The retention of volatile compounds in orange juice is shown in Figure 2. A gap in the vicinity of 70% can be seen in the figure, which divided the 8 compounds into 2 classes. Ethyl butyrate, *trans*-2-hexenol, α -pinene, phellandrene, and limonene were retained to a lesser extent, while linalool, nonanal, and citronellol were retained more effectively; furthermore, nonanal and citronellol were obtained in higher content than the untreated sample (100%) with DPCD duration times beyond 30 min. In contrast, the contents of the monocyclic terpenes exhibited the lowest values after 30 or 40 min of DPCD treatment. Compared with the DPCD-treated samples, the contents of the volatile compounds in the heat-treated sample (Supplemental data Figure S1) were near the values of the DPCD samples after 60 min duration ($p > 0.05$), except those of *trans*-2-hexenol and citronellol, which showed lower levels ($p < 0.05$).

3.3. Linear Regression Analysis. The changes of the parameters with extending DPCD treatment time were linearly fitted, and results are shown in Table 1. $D(4,3)$, L^* , a^* , ethyl butyrate, and *trans*-2-hexenol decreased linearly ($p < 0.05$), while b^* , α -pinene, phellandrene, limonene, linalool, and citronellol were not linearly changed. Nonanal was the only volatile component which increased linearly. It is worth noting that the L^* values decreased linearly, although these were not significantly different when using ANOVA.

3.4. Correlation Analysis. The interdependence of the physical and volatile variables was investigated by analyzing their correlation coefficients (Table 2). The particle size $D(4,3)$ was significantly correlated ($p < 0.05$) with the ethyl butyrate ($R = 0.822$) and citronellol ($R = -0.821$), indicating that a high particle size value was always associated with a high ethyl butyrate concentration but with a low content

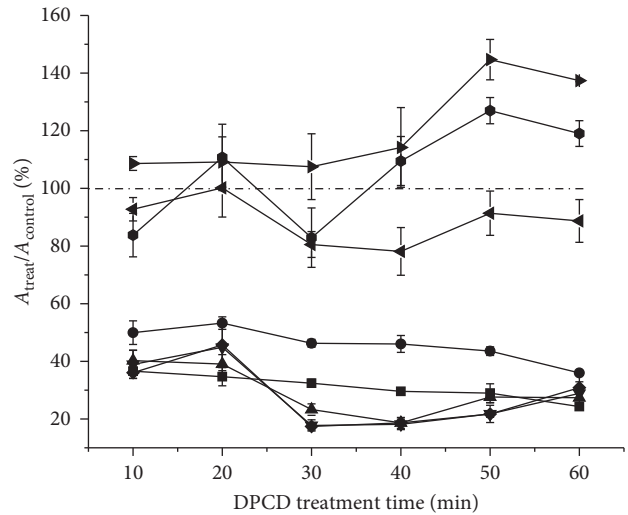


FIGURE 2: Effect of DPCD processing (40 MPa, 55°C) on the volatile components in orange juice during 60 min: ►, nonanal; ●, citronellol; ◄, linalool; ◆, *trans*-2-hexenol; ■, ethyl butyrate; ▼, α -pinene; ▲, phellandrene; ◆, limonene. Each value represents the mean \pm standard deviation of three replicates, and A in the y -axis denotes the peak area of the volatile compounds, taking the average value of the untreated sample as control.

TABLE 1: Linear regression analysis of DPCD treatment time on 12 parameters of orange juice.

	Regression equation	R	p
$D(4,3)$	$y = 99.18 - 0.466x$	-0.8639	0.027*
L^*	$y = 100.38 - 0.006x$	-0.9729	0.001**
a^*	$y = 88.59 - 0.338x$	-0.9648	<0.001***
b^*	$y = 108.22 - 0.0285x$	-0.6057	0.203
Ethyl butyrate	$y = 39.230 - 0.233x$	-0.9843	<0.001***
<i>trans</i> -2-Hexenol	$y = 55.762 - 0.284x$	-0.8971	0.015*
α -Pinene	$y = 39.695 - 0.295x$	-0.6402	0.171
Phellandrene	$y = 40.306 - 0.3405x$	-0.5608	0.247
Limonene	$y = 38.070 - 0.276x$	-0.4622	0.356
Linalool	$y = 93.531 - 0.140x$	-0.3208	0.535
Nonanal	$y = 94.570 + 0.734x$	0.8369	0.038*
Citronellol	$y = 80.346 + 0.719x$	0.7355	0.096

Note. y represents the relative value and x represents the DPCD treatment time from 10 to 60 min; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

of citronellol. L^* showed no significant correlations with any other parameters. The a^* value showed a significant positive correlation with the ethyl butyrate ($R = 0.961$, $p = 0.002$) and *trans*-2-hexenol ($R = 0.888$, $p = 0.018$) but a significant negative correlation with the content of nonanal ($R = -0.873$, $p = 0.023$). The value of b^* was also positively correlated with the content of α -pinene ($R = 0.997$, $p < 0.001$), phellandrene ($R = 0.934$, $p = 0.006$), and limonene ($R = 0.888$, $p = 0.018$). There were also significant positive correlations between the contents of some volatile compounds. Linalool was significantly correlated ($p < 0.05$) with the content of α -pinene, phellandrene, and limonene, and the three monoterpenes were significantly positively correlated with each other.

TABLE 2: Correlation coefficient matrix of 12 parameters measured in DPCD-treated orange juice samples.

	<i>D</i> (4, 3)	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	Ethyl butyrate	<i>trans</i> -2- Hexenol	α -Pinene	Phellandrene	Limonene	Linalool	Nonanal	Citronellol
<i>D</i> (4, 3)	1.000											
<i>L</i> [*]	-0.303	1.000										
<i>a</i> [*]	0.770	-0.470	1.000									
<i>b</i> [*]	0.375	0.042	0.496	1.000								
Ethyl butyrate	0.822 *	-0.552	0.962 **	0.602	1.000							
<i>trans</i> -2- Hexenol	0.631	-0.667	0.888 *	0.522	0.928 **	1.000						
α -Pinene	0.415	0.068	0.537	0.997 ***	0.627	0.531	1.000					
Phellandrene	0.294	0.199	0.453	0.934 **	0.512	0.504	0.939 **	1.000				
Limonene	0.169	0.265	0.365	0.888 *	0.409	0.436	0.892 *	0.990 ***	1.000			
Linalool	-0.013	0.077	0.244	0.916	0.345	0.373	0.891 *	0.882 *	0.887 *	1.000		
Nonanal	-0.741	0.297	-0.873 *	-0.180	-0.771	-0.746	-0.238	-0.281	-0.221	0.088	1.000	
Citronellol	-0.821 *	0.149	-0.781	-0.129	-0.680	-0.447	-0.196	-0.075	0.024	0.231	0.809	1.000

*, **, and *** in bold indicated significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Correlation coefficients are based on Pearson's test.

The associations and differences in the parameters can be clarified by a simple inspection of the heat map (Figure 3): the levels of some parameters increased during processing (group I, nonanal and citronellol), while others decreased (group II, *D* (4, 3), *L*^{*}, *trans*-2-hexenol, ethyl butyrate, and *a*^{*}). The levels of the parameters in group III (linalool, phellandrene, limonene, α -pinene, and *b*^{*}) were also closely related, decreasing within 40 min of DPCD processing and then increasing after 50 and 60 min treatment to intermediate values between those for 10–20 min and 30–40 min treatments.

The results can be summarized by PCA (Figure 4). The two major components accounted for 90.5% of the cumulative variance. The PC1 (horizontal) explains 61.8% of the variance; on this axis, the samples were clearly arranged from right to left according to their DPCD duration time on the score plot (Figure 4(a)). PC2 (28.7%) separated the samples with medium treatment time (30 and 40 min) from the other four samples and all of the samples forming a “U” shape on the dimension plot. The PCA clustered the parameters in the same manner as the CA results in Figure 1, but the PCA plot showed opposite directions of group I and group II, with group III settled between them. When the data from the original fresh sample and the heat-treated sample were involved in PCA plots, limonene, linalool, α -pinene, phellandrene, and *a*^{*} were close to the fresh sample, which indicated that the higher values of these parameters represented the characteristics of fresh orange juice, while the changes in the nonanal and citronellol contents, as well as the *b*^{*} values, were correlated with the longer DPCD treatments. In addition, the samples with DPCD treatment time below 20 min were closer to the original fresh sample than the heat-treated sample on PC1 (Figure 5), and the increasing trend of *L*^{*} values was generally correlated with the heat-treated sample.

4. Discussion

Multivariate analysis is a powerful method to extract hidden information from a large amount of data. It can generate visual graphs or plots, and it has been used widely to elucidate

the inner relationships/differences between food structures, quality, processing procedures, etc. [17]. It is well known that the heat treatment of orange juice generally causes significant changes, while DPCD-treated samples usually present some advantages in quality retention [3, 18]. CA and PCA made it possible to illuminate the differences of DPCD-treated samples from the untreated and heat-treated samples. In the present study, CA and PCA were used to reveal the association among the parameters relevant to the quality of orange juice processed by DPCD. Indeed, CA and PCA helped to group these parameters according to the three different ways in which they changed with lengthening DPCD duration.

Most researchers considered that DPCD treatment could lead to smaller particles in the juice colloid, probably by means of the homogenization effect, which facilitates the splitting of larger particles into smaller ones [14, 19, 20], while Zhou et al. [15] found that the particle size increased in carrot juice and peach juice as a result of acid-induced protein coagulation. However, the particle size in this study was not successively decreased. Most probably, this change was the combined outcome of both CO₂-induced coagulation and DPCD-induced homogenization, among which the homogenization effect should be the major effect, although it required some time to be obviously manifested. Otherwise, the overall reduction of particle size by DPCD treatment might indicate the stability enhancement and quality improvement of the juice [9].

According to the reports on color changes in citrus juices after DPCD treatment, DPCD increased the yellowness values of carrot juice [14], orange juice [19], and mandarin juice [16] while decreasing their redness values. Our results in the present study confirmed these changes. Some other treatments such as heat treatment could also result in similar changes in color. For example, Lee and Coates [21] found the same color changes in orange juice after pasteurization when *a*^{*} value decreased, while *b*^{*} increased significantly. They considered that the isomerization of 5,6-epoxide carotenoids to 5,8-epoxide carotenoids was probably responsible for the color changes. However, Arena et al. [22] provided the insight that the varying distribution of carotenoids between the serum and the pulp, as well as the modification of

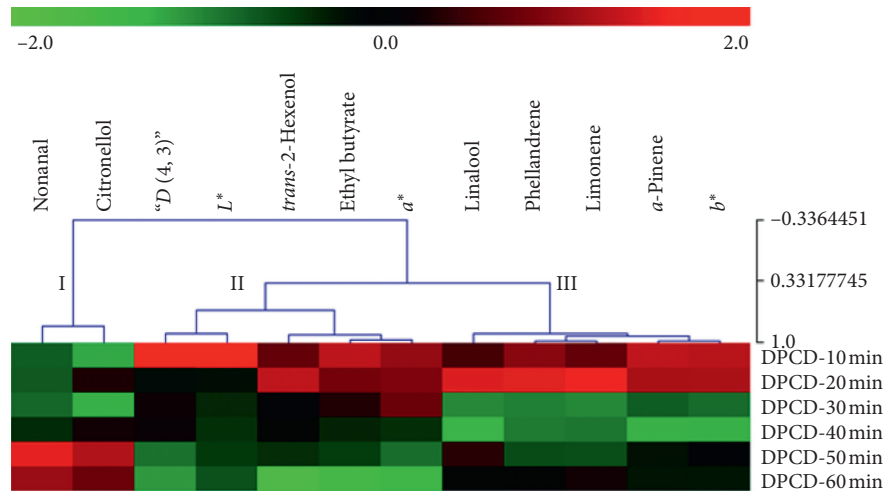


FIGURE 3: Dendrogram of cluster analysis of the 12 parameters of the DPCD-treated juices (40 MPa, 55°C). All values in the heat map were transformed into Z-scores and clustered with Euclidean distances.

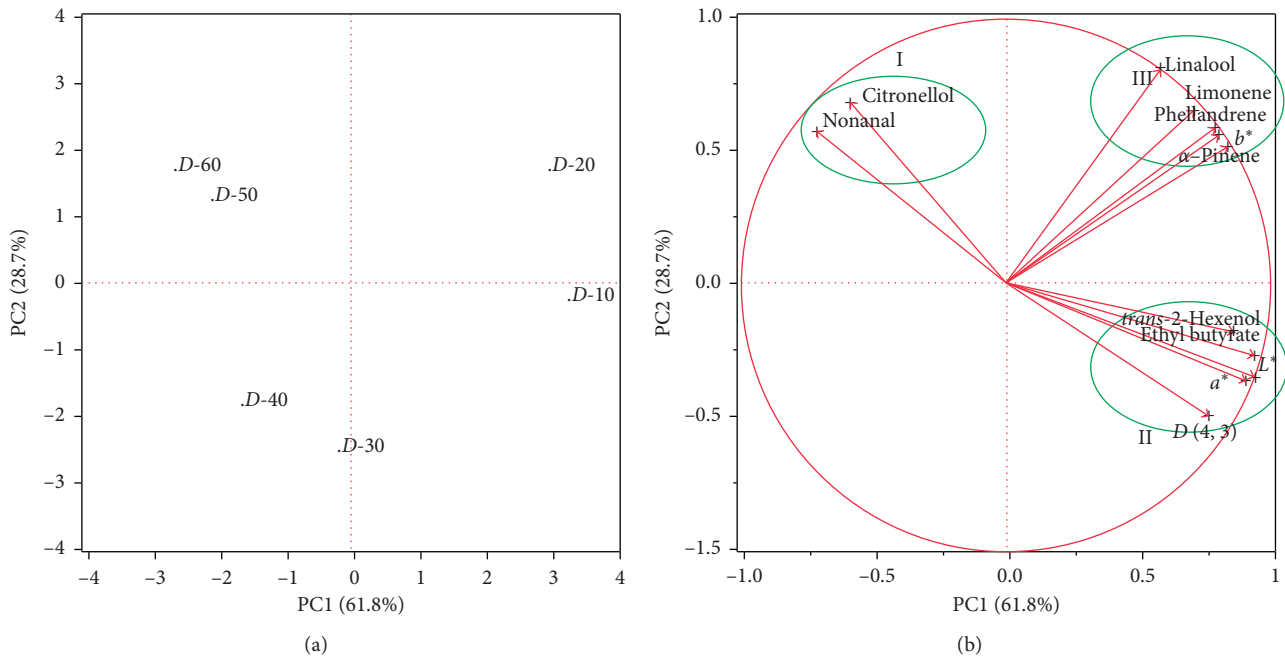


FIGURE 4: PCA plots for changes in quality of orange juice during DPCD treatment (40 MPa, 55°C). (a) PCA scores of the samples; (b) PCA loadings of the parameters. *D*-10, 20, 30, 40, 50, and 60 indicate DPCD duration times of 10, 20, 30, 40, 50, and 60 min, respectively.

particles, could be responsible for the change of the orange juice color; Kraska et al. [11] reported that the solubility of β -carotene, a low-solubility pigment in compressed CO_2 , depended on the density of the supercritical fluid which was an outcome of pressure and temperature [12]. In the present study, the increased yellowness and the decreased redness might be attributed to the comprehensive effects of the distribution change and isomerization of carotenoids as well as the modification of particles.

The volatile components of orange juice are partitioned between the insoluble pulp and the aqueous serum. The insoluble components in orange juice were classified as pulp particles ($>2\ \mu\text{m}$) and finer particles ($<2\ \mu\text{m}$) [19, 23]. The

monoterpene, sesquiterpene, and long chain aliphatic aldehydes existed primarily in the orange juice pulp, while esters and monoterpene alcohols were mainly in the serum [24]. In this study, the mild increase of three monoterpenes (limonene, phellandrene, and α -pinene) and nonanal in the DPCD-treated samples with duration times longer than 40 min might be due to the splitting effect of the coarsest particles ($>2\ \mu\text{m}$), since they were primarily in pulp. However, ethyl butyrate and *trans*-2-hexenol decreased linearly with time extension of DPCD treatment, indicating that they were mainly present in the serum and unable to be enhanced by the DPCD effect. Considering the lower level of the volatile compounds after either the heat treatment or the

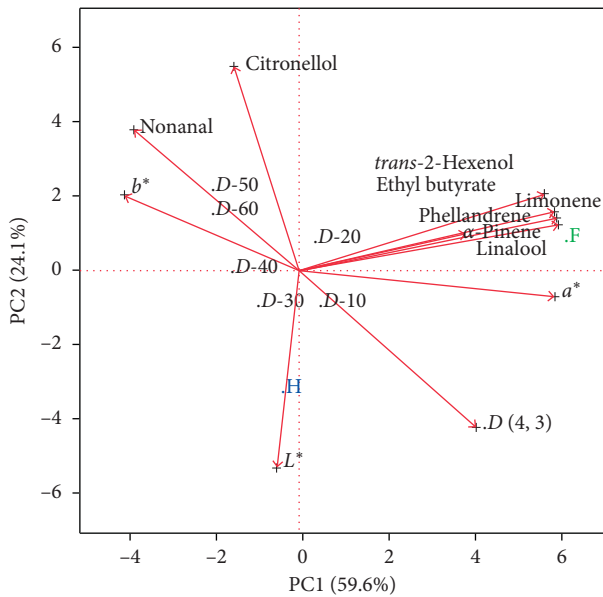


FIGURE 5: PCA plots comparing the DPCD-treated, fresh untreated, and heat-treated orange juices. *D*-10, 20, 30, 40, 50, and 60 indicate DPCD durations of 10, 20, 30, 40, 50, and 60 min, respectively, at 55°C and 40 MPa. *F*: untreated freshly squeezed juice sample; *H*: heat-treated (90°C, 60 s) juice sample.

DPCD treatment compared with the control, the declining trend of some volatile components might be attributed to their volatility, the effect of heat, and their release during depressurization [25, 26].

The juice quality also depends on the volatile compounds. In fresh orange juice, fewer than 25 volatile components are odor-active [10]. *trans*-2-Hexenol arises from 3-hexenal, which is an important contributor to the green, grassy top-note of freshly prepared orange juices, which is nearly always reduced significantly in processed juices [27]. The DPCD-treated samples in this study retained significantly more *trans*-2-hexenol than the heat-treated one, possibly indicating that a higher intensity of fresh odor remained. Ethyl butanoate is one of the most potent esters in orange products and is described as a fruity flavor [10]. Linalool is the most aroma intensive alcohol in orange juice and possesses a distinctive floral, sweet odor [10]. Both ethyl butanoate and linalool are important contributors to the desirable aromas. Linalool is less sensitive to the DPCD and heat treatment without significant change. Additionally, ethyl butanoate was reduced linearly with DPCD treatment time extension, and the samples with the longer treatment times (50 and 60 min) showed similar content to the heat-treated one, suggesting a decrease of fruit aroma intensity.

Limonene is the most abundant component, usually with a peak area over 90% of the total area when determined using GC [28] but is readily converted into α -terpineol and carvone which are indicators of the aroma degradation of orange juice during processing and storage [29]. Limonene and α -pinene contribute to the minty, lemon, citrus-like and resinous, pine tree, ethereal odor of orange juice, respectively [10]. The aliphatic saturated aldehyde nonanal contributes a significant odor component, described as metallic, to orange

essence oils [30], but a citrus-like, soapy, floral character in juice [10]. Citronellol and α -pinene are aroma components with minor content in orange juice [30], contributing floral, sweet, metallic, and minty odors [31].

Nonanal and the monoterpenes nearly always demonstrate higher content in mechanically extracted juice than in hand-squeezed samples, since they are correlated with pulp content [24] and peel oil [10]. The reduction of ethyl butanoate and the monoterpenes, as well as the increase of nonanal, was reported in high hydrostatic pressure-processed orange juice, while the aroma characteristics of HHP-treated juice were unchanged [27]. Therefore, the changes of the flavor component concentrations in orange juice possibly do not significantly modify the sensory aroma; nevertheless, extended treatment should be avoided in order to retain the fresh flavor.

Most previous studies only reported the reduction of volatile compounds after DPCD [3–5]; in this work, increases of some volatile compounds were detected, and the increase of the volatile components was found to be due to the decreasing changes of particles. In addition, the high correlations of the volatile components and color parameters might reflect that the volatile components and pigments changed simultaneously in the juice system.

5. Conclusion

A major challenge for the juice processing industry is to produce a juice with a long shelf life and a flavor close to freshly squeezed juice. Here, the DPCD-treated samples with duration times below 20 min could be closer to the freshly squeezed than the heat-treated sample. The CA and PCA were powerful tools to help assess the changes in the orange juice samples. The changes in particle size, color, and volatile components of orange juice during DPCD processing were significantly correlated. The changes in the orange juice indicated that DPCD offers the potential to be used to produce juice products of excellent quality.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Figure S1. Effect of DPCD processing on volatile components of orange juice: (A) ethyl butyrate; (B) *trans*-2-Hexenol; (C) α -pinene; (D) phellandrene; (E) limonene; (F) linalool;

(G) nonanal; (H) citronellol. Different letters on the columns indicate significant differences ($p < 0.05$). *F*, freshly squeezed juice; 1–6, DPCD-treated samples with time of 10, 20, 30, 40, 50, and 60 min, respectively; *H*, heat-treated sample. (*Supplementary Materials*)

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Research Article

High-Voltage Electric Field-Assisted Thawing of Frozen Tofu: Effect of Process Parameters and Electrode Configuration

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Applying high-voltage electric field (HVEF) to some food materials has been shown to increase the thawing rate. To investigate the effect of process parameters and electrode configuration in high-voltage electric field system, we took the frozen tofu as the research object and investigated the influence of the different voltages, electrode configuration, and electrode distances on thawing process. The thawing time, center temperatures, and loss rate of samples were measured. The results showed that the thawing time of frozen tofu decreases with the increase of voltage and the thawing time has a great relevance with configuration and distance of electrodes. The electric parameters have a major effect on thawing loss and thawing time when center temperatures of frozen tofu are from -2°C to 0°C . This work provides clues and experimental basis for the further application of high-voltage electric field thawing technology.

1. Introduction

Thawing of frozen materials is an important component of food product processing. Many technologies are widely used in food thawing industry, such as cold and warm water thawing, still air thawing, refrigerator thawing, and so on. However, all have their own shortcomings, which greatly affects the products' subsequent production and market competitiveness, including higher color deterioration and weight loss, longer thawing time, and decreased nutritional value of the thawed products. Therefore, it is significant to explore the new thawing technology.

High-voltage electric field (HVEF) thawing is an important nonthermal food processing method that has received considerable attention lately [1], and it is predominantly used to thaw food products in academia, including pork [2, 3], tuna fish [4–7], chicken [8], rabbit meat [9], shrimps [10], common carp [11], tofu [12], and apple tissue [13]. Compared to traditional thawing methods, high-voltage electric field thawing has many advantages [3, 6, 10] such as reduced thawing time, food quality preservation, microbial growth

inhibition, reduced energy consumption, and so on. The thawing can be carried out using either AC or DC high voltages. Multipoint and plate electrode systems are efficient in accelerating thawing of freeze materials [10]. When the thawing temperature was set at -3°C , the thawing time of frozen chicken under high-voltage electric field was $2/3$ the time taken for thawing meat [14]. The HVEF treatment significantly shortened the thawing time of frozen pork tenderloin meat, and thawing time was reduced to $2/3$ that of the control and reduced the total microbial counts in thawed frozen meat by $0.5\text{--}1$ log CFU/g [2]. Mousakhani-Ganjeh et al. reported that the high-voltage electric field could increase susceptibility of tuna fish to lipid oxidation due to ozone generation [6]. In previous studies, we studied the thawing characteristic of frozen tofu from the thawing rate, center temperatures, thawing loss, specific energy consumption, and mathematical models under AC electric field at different voltages and found that the thawing rate of frozen tofu was notably greater in the high-voltage electric field system when compared to control [12]. Both linear and quadratic models were the best mathematical models [12].

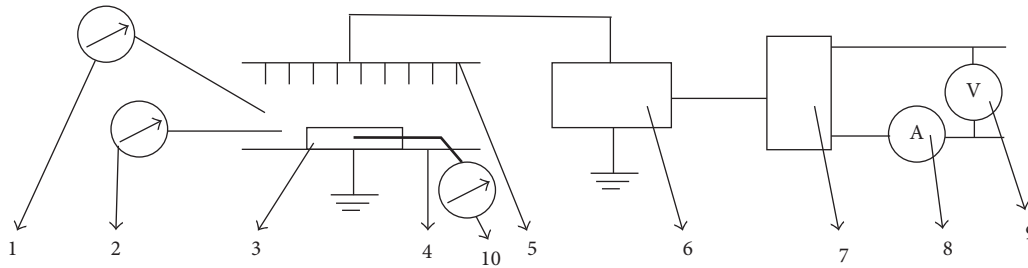


FIGURE 1: Schematic diagram of HVEF thawing. 1: thermometer; 2: hygrometer; 3: frozen tofu; 4: grounded plate electrode; 5: needle electrodes; 6: high-voltage power source; 7: voltage regulator; 8: amperometer; 9: voltmeter; 10: temperature sensor.

Despite the detail with which these studies treat the problem, few studies have systematically and comprehensively reported on the effects of process parameters and electrode configuration under different high-voltage electric field thawing conditions.

To further investigate the effect of process parameters and electrode configuration for optimizing and improving the thawing efficiency in high-voltage electric field system, we studied the thawing characteristic of frozen tofu. To accomplish this, high-voltage electric field thawing characteristics and quality of frozen tofu were studied, including the thawing electric voltage, thawing time, center temperatures of samples, thawing loss, and the configuration of the electrodes under different thawing conditions.

2. Materials and Methods

2.1. Experimental Facility. The thawing experimental equipment is shown schematically in Figure 1 [12, 15]. The HVEF thawing equipment consists of three parts: high-voltage power system, thawing system, and control system. The thawing system consists of a high-voltage electrode, the configuration of which is multiple sharp pointed needles or wires or metallic plate, and a fixed horizontal grounded metallic plate. The electrode gaps between the high-voltage electrodes and the grounded electrode can be adjusted. The high-voltage electrodes were connected to a high-voltage power system that can supply alternating current (AC) or direct current (DC) high voltage. The frequency of the AC electric field was 50 Hz. The high-voltage power (YD(JZ)-1.5/50, made in Wuhan, China) was connected to a voltage regulator (KZX-1.5 KVA, made in Wuhan, China) as control system, with an adjustable voltage ranging from 0 to 50 kV for AC or 0 to 70 kV for DC by a controller. The grounded electrode was an 80 cm × 40 cm rectangular stainless steel plate. The ambient temperature and relative humidity in thawing process were both measured. The voltage and current of HVEF system were measured by a voltmeter (made in Ningbo, China) and an amperometer (made in Ningbo, China), respectively. The metal needle which the length is 20 mm and diameter is 1 mm was evenly arranged on the needle plate electrode (64 cm × 40 cm) by stainless steel wire. The distance between two needle electrodes was 40 mm. The dimensions of high-voltage electrode with plate or wires were a 64 cm × 40 cm. The distance between two stainless steel wires was 40 mm. All the samples were spread

in a single layer on the grounded plate electrode at random. The center temperature of samples was measured by a temperature sensor.

2.2. Preparation and Treating of Tofu. The soft tofu was purchased from a local market near Inner Mongolia University of Technology, Hohhot, China. The fresh soft tofu was sliced into sheet about 3.5 cm × 3.5 cm × 3.5 cm using a knife and immediately frozen at -18°C in a refrigerator (Hisense BCD-197T, made in Qingdao, China). The frozen samples were stored at -18°C until use.

2.3. Measurement of Thawing Process and Thawing Time. We conducted three experimental conditions to further investigate the effect of process parameters and electrode configuration for optimizing and improving the thawing efficiency in high-voltage electric field system. The voltage, electrode configuration, and discharge gap were investigated, respectively.

Firstly, the frozen tofu was thawed under DC electric field with multiple needles-to-plate electrode at different voltages. The thawing voltage was 0 kV (the control samples), 4 kV, 8 kV, 12 kV, 16 kV, 20 kV, 24 kV, or 28 kV, respectively. The corresponding discharge gap between the high-voltage electrodes and the grounded electrode was 100 mm.

Secondly, the configuration of high-voltage electrodes was multiple needles-to-plate, multiple wires-to-plate, or multiple plate-to-plate electrodes, respectively, under AC electric field. The corresponding discharge gap between the high-voltage electrodes and the grounded electrode was 100 mm. The corresponding voltage was 28 kV. The same thawing experiments were also investigated under DC electric field.

Lastly, the discharge gap between the emitting point and the grounded electrode each time at 8 cm, 9 cm, 10 cm, 11 cm, and 12 cm with multiple needles-to-plate electrode for AC electric field was changed, and the corresponding voltage was 20 kV. The sample thickness is lower than the discharge gap to make better use of the nonuniform electric field and the ion wind. And this would better reduce energy consumption during thawing.

The thawing temperature was $20 \pm 1^{\circ}\text{C}$, the relative humidity was $30 \pm 5\%$, and the ambient wind speed was 0 m/s. The center temperature of frozen tofu samples determined by a digital thermometer (made in China) and recorded at 5 min intervals during the thawing process. In

this paper, “center temperature” refers to the temperature at the geometric center of frozen tofu samples, where heat takes longest to penetrate. Thawing was continued until the center temperature of the frozen tofu sample reached 10°C. The time required to raise the center temperature from −10°C to 10°C was determined as thawing time. Thawing experiments were independently performed three times in this study and the average was taken. In each thawing experiment, the sample is placed at the same position on the grounded electrode to keep the consistency of the experiment. It is found in other studies that both the center and surface temperatures of samples increased rapidly with the increase of applied voltages and have some similar characteristics [3]. So, we only measured the center temperature of frozen tofu to investigate the effect of process parameters and electrode configuration.

There are some electrochemical reactions during the thawing process, which may lead to electrode corrosion and metal release. When the electrode was designed, we use

a protective paint on the surface to prevent electrode oxidation and corrosion. And there is no contact here between the electrode and the material during the thawing process. Then, the effect of electrochemical reaction on the thawing process is very small. We think that it can be ignored.

The thawing rate of material samples (g/s) was calculated using the following equation:

$$\text{thawing rate} = \frac{\text{weight of frozen tofu (g)}}{\text{thawing time of frozen tofu (s)}}. \quad (1)$$

2.4. Measurement of Evaporation Loss, Thawing Loss, and Drip Loss. Evaporation loss, thawing loss, and drip loss were measured by weighing the frozen and thawed material samples before and after the removal of surface water according to (2), (3), and (4), respectively:

$$\text{evaporation loss} = \frac{(\text{weight of the frozen tofu}) - (\text{weight of the thawed tofu before removing surface water})}{\text{weight of the frozen tofu}}, \quad (2)$$

$$\text{thawing loss} = \frac{(\text{weight of the frozen tofu}) - (\text{weight of the thawed tofu after surface water removal})}{\text{weight of the frozen tofu}}, \quad (3)$$

$$\text{drip loss} = \text{thawing loss} - \text{evaporation loss}. \quad (4)$$

Experiments were independently performed three times in this study and the average was taken.

2.5. Statistical Analysis. Single-factor analysis of variance was used to calculate the evaporation loss, thawing loss, and drip loss between the frozen tofu under alternating electric field and without electric field (control). The evaporation loss, thawing loss, and drip loss between different electric field were also calculated using single-factor analysis of variance. The differences in thawing loss are considered statistically significant when $p < 0.05$.

3. Results and Discussion

3.1. Thawing Time and Thawing Rate Analysis. Figure 2(a) shows the effect of different voltages on the thawing time and thawing rate under DC electric field. The frozen tofu was thawed at 20°C with a fixed discharge gap of 10 cm under DC electric field. The thawing times at 0 kV (the control), 4 kV, 8 kV, 12 kV, 16 kV, 20 kV, 24 kV, and 28 kV under DC electric field were 200 min, 155 min, 150 min, 145 min, 140 min, 135 min, 130 min, and 125 min, respectively. In other words, the thawing times treated with the high-voltage electric field were significantly shortened than those of the control (0 kV). With the increase of voltage, the

thawing time decreased. The high-voltage electric field could obviously accelerate the thawing rate of tofu samples compared to that of the control, and increasing the voltage had a major effect on the enhancement of the thawing rate. These results agree with those studies which reported enhancement in thawing rate with increase of applied voltage [3, 4]. At present, it is generally believed that the main reason of thawing rate accelerating is the generation of corona wind which was produced by the high-voltage electric field. The samples are put on a metal plate (cathode), while electrodes are mounted in some distance to the cathode and will form a corona when the electric circuit is closed [16]. Under the HVEF system, the corona wind produced impinges on the material and disturbs the liquid part of the thawing tofu, leading to thawing enhancement. The corona electrode with small curvature radius could form a nonuniform HVEF and realize a corona discharge in the gas-filled gap. The corona wind would be higher when the configuration of the electrodes is adopted in the form of needle-to-plate or wire-plate rather than the plate-to-plate form. Thus, the configuration of the electrodes would impact on the thawing rate of frozen food.

Figure 2(b) shows the effect of different configuration of the electrodes on the thawing time and thawing rate. The thawing times for the electrodes of plate-to-plate under DC

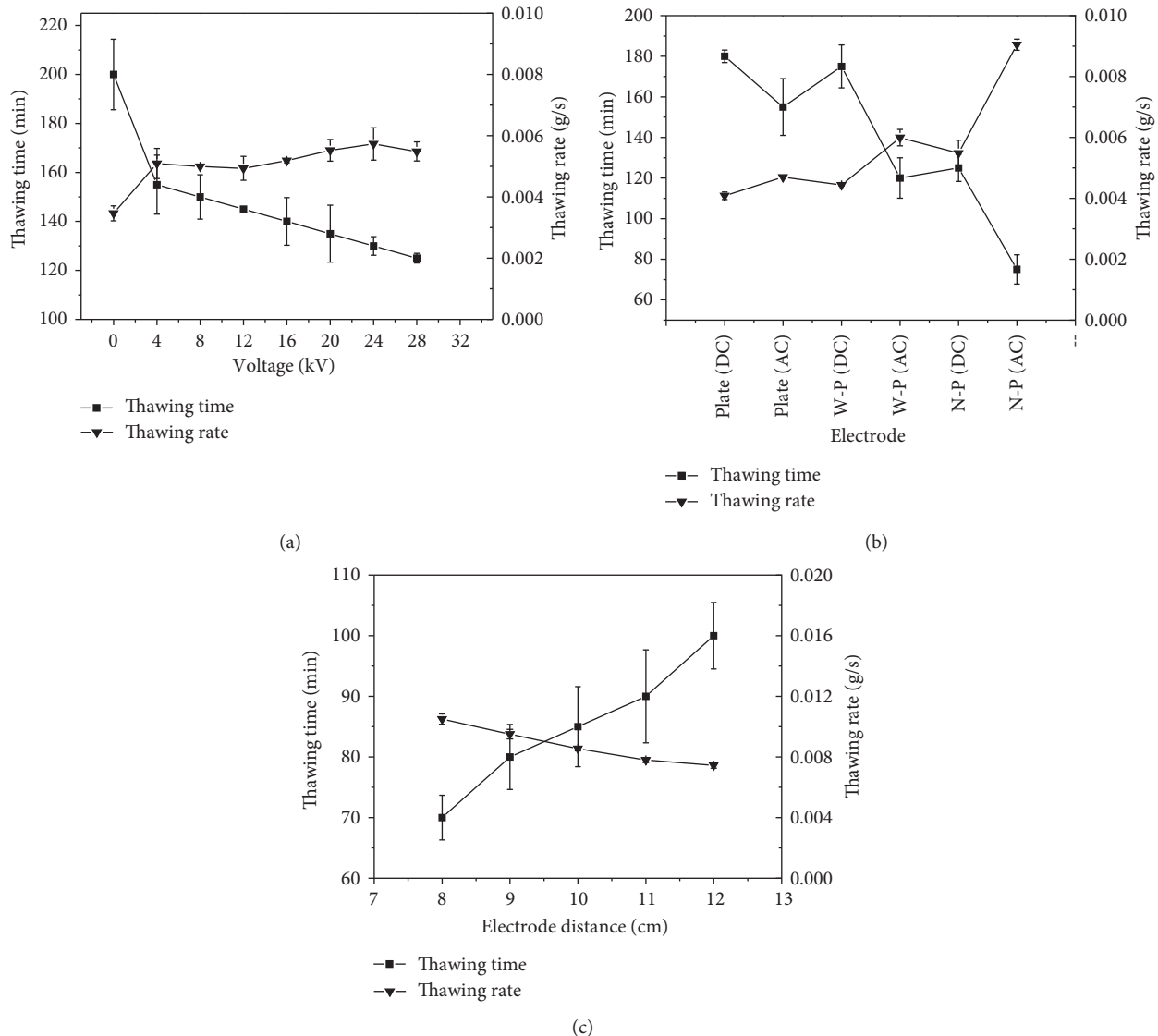


FIGURE 2: (a) Thawing time and thawing rate of frozen tofu in different voltages. The frozen tofu was thawed at 20°C with a fixed discharge gap of 100 mm under DC electric field. The thawing voltage was 0 kV (the control samples), 4 kV, 8 kV, 12 kV, 16 kV, 20 kV, 24 kV, or 28 kV, respectively. (b) Thawing time and thawing rate of frozen tofu in different electrodes. Plate (DC): the plate-to-plate electrodes under DC electric field, plate (AC): the plate-to-plate electrodes under AC electric field, W-P (DC): the wires-to-plate electrodes under DC electric field, W-P (AC): the wires-to-plate electrodes under AC electric field, N-P (DC): the needles-to-plate electrodes under DC electric field, and N-P (AC): the needles-to-plate electrodes under AC electric field. The corresponding discharge gap between the high-voltage electrodes and the grounded electrode was 100 mm. The corresponding voltage was 28 kV (c) Thawing time and thawing rate of frozen tofu in different electrode distances. The discharge gap was 8 cm, 9 cm, 10 cm, 11 cm, and 12 cm with multiple needles-to-plate electrode for AC electric field, respectively. The corresponding voltage was 20 kV.

electric field, plate-to-plate under AC electric field, wires-to-plate under DC electric field, wires-to-plate under AC electric field, needles-to-plate under DC electric field, and needles-to-plate under AC electric field were 180 min, 155 min, 175 min, 120 min, 125 min, and 75 min, respectively. We can see that the thawing time under AC electric field was shortened significantly when compared to that under DC electric field. The thawing time for the electrodes of needles-to-plate under AC electric field was the shorter than that under the other experimental conditions. And the thawing time for the electrodes of plate-to-plate

under DC electric field was the longer than that under the other experimental conditions. The thawing rate of tofu samples treated with AC electric field is higher than that treated with DC electric field. From Figure 2(b) shows that the thawing rate of frozen tofu under AC electric field is higher than that under DC electric field when they have the same voltage and electrodes. Therefore, another high-voltage electric field thawing mechanism is possible besides corona wind under the AC electric field. As water molecules are highly polar, they orient themselves in the direction of the electric field, which in-turn would lead to the conversion of

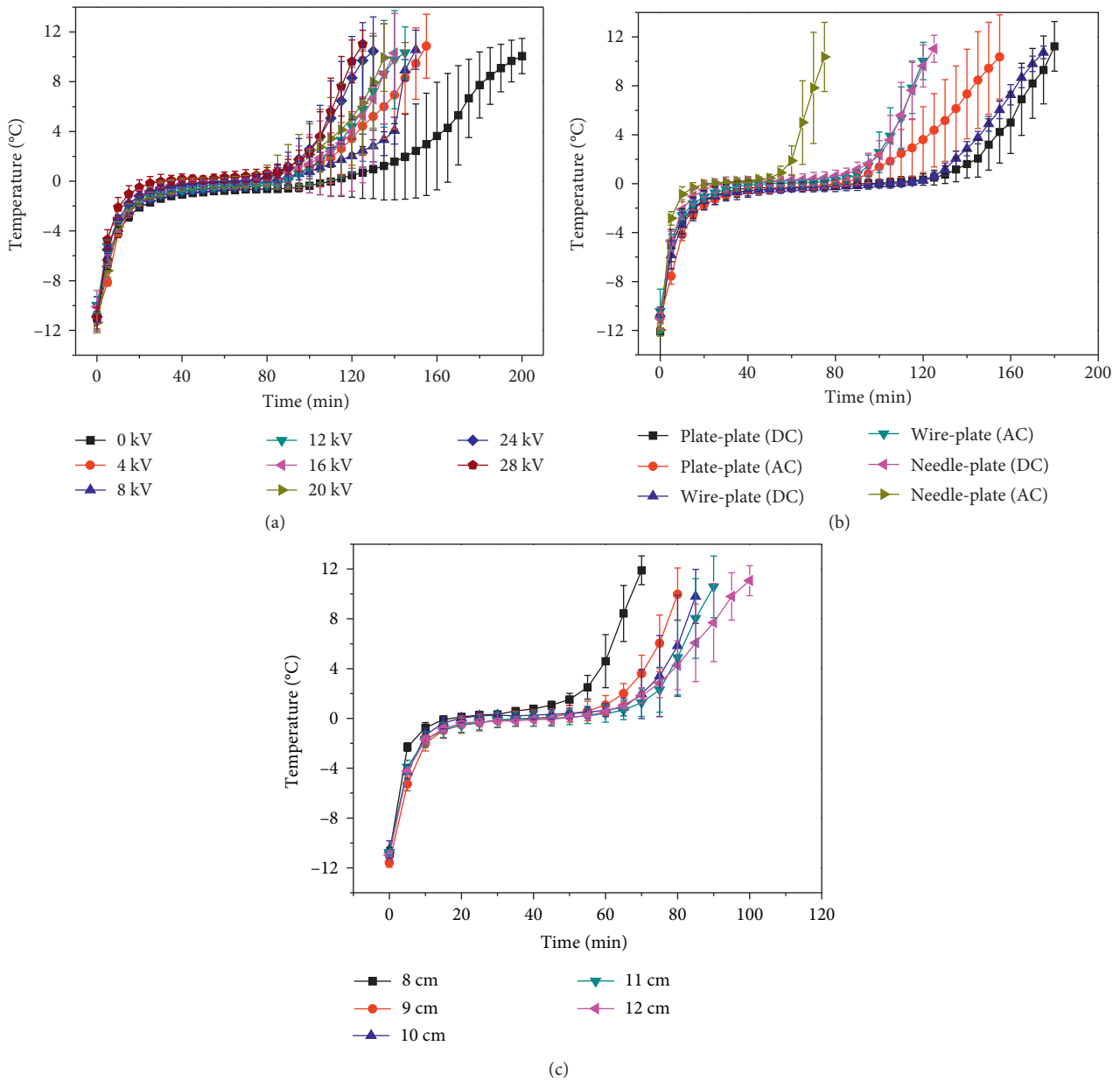


FIGURE 3: (a) Changes in temperature during thawing of frozen tofu in different voltages. The frozen tofu was thawed at 20°C with a fixed discharge gap of 100 mm under DC electric field. The thawing voltage was 0 kV (the control samples), 4 kV, 8 kV, 12 kV, 16 kV, 20 kV, 24 kV, or 28 kV, respectively. (b) Changes in temperature during thawing of frozen tofu in different electrodes. The corresponding discharge gap between the high-voltage electrodes and the grounded electrode was 100 mm. The corresponding voltage was 28 kV. (c) Changes in temperature during thawing of frozen tofu in different electrode distances. The discharge gap was 8 cm, 9 cm, 10 cm, 11 cm, and 12 cm with multiple needles-to-plate electrode for AC electric field, respectively. The corresponding voltage was 20 kV.

electrical energy into mechanical energy, thereby forcing water molecules out of the material [12]. Relevant study also showed that the high field intensity can reorient water molecules in ice and modify the crystal morphology, so that freezing is inhibited, leading to the acceleration of the thawing process [17].

Figure 2(c) that the effect of different discharge gaps on the thawing time and thawing rate. The thawing times for the discharge gaps of 8 cm, 9 cm, 10 cm, 11 cm, and 12 cm were 70 min, 80 min, 85 min, 90 min, and 100 min, respectively. As discharge gap increased, the thawing time increased.

These results are similar with what has been found in other studies [3, 4]. The electric field strength decreases with the increase of discharge gap. The magnitude of the electric wind velocity was proportional to the electric field strength. So, decreasing the discharge gap had a major effect on the enhancement of the thawing rate.

3.2. Center Temperatures Analysis. The center temperatures exposed to high-voltage electric field are shown in Figure 3. The results indicate that within the first 10 min, the center

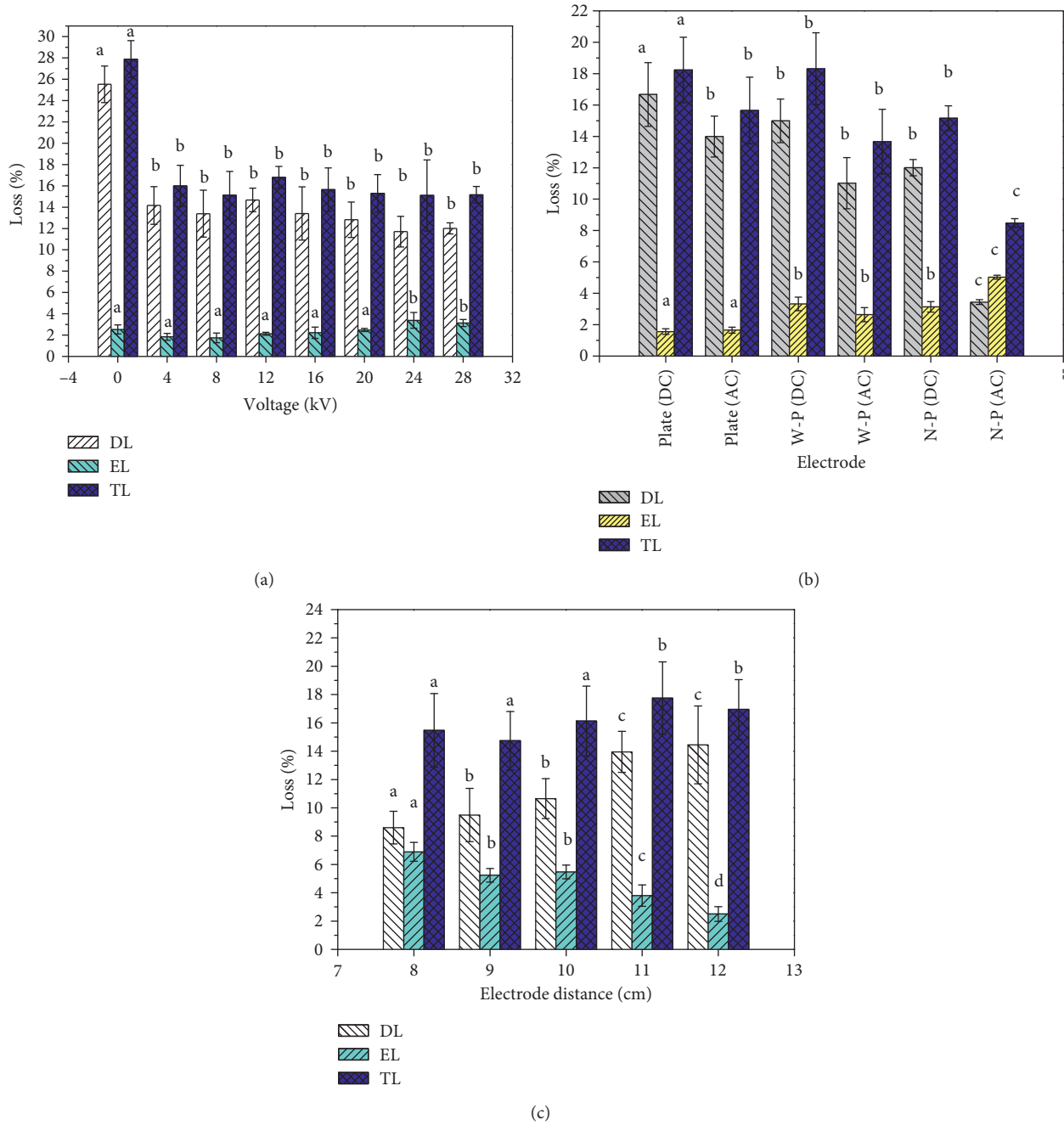


FIGURE 4: (a) The evaporation loss, thawing loss, and drip loss of tofu under different voltages. The frozen tofu was thawed at 20°C with a fixed discharge gap of 100 mm under DC electric field. The thawing voltage was 0 kV (the control samples), 4 kV, 8 kV, 12 kV, 16 kV, 20 kV, 24 kV, or 28 kV, respectively. For each treatment, means with different lower case letters are significantly different ($p < 0.05$). (b) The evaporation loss, thawing loss, and drip loss of tofu under different electrodes. Plate (DC): the plate-to-plate electrodes under DC electric field, plate (AC): the plate-to-plate electrodes under AC electric field, W-P (DC): the wires-to-plate electrodes under DC electric field, W-P (AC): the wires-to-plate electrodes under AC electric field, N-P (DC): the needles-to-plate electrodes under DC electric field, and N-P (AC): the needles-to-plate electrodes under AC electric field. The corresponding discharge gap between the high-voltage electrodes and the grounded electrode was 100 mm. The corresponding voltage was 28 kV. For each treatment, means with different lower case letters are significantly different ($p < 0.05$). (c) The evaporation loss, thawing loss, and drip loss of tofu under different electrode distances. The discharge gap was 8 cm, 9 cm, 10 cm, 11 cm, and 12 cm with multiple needles-to-plate electrode for AC electric field, respectively. The corresponding voltage was 20 kV. For each treatment, means with different lower case letters are significantly different ($p < 0.05$).

temperatures of frozen tofu increased rapidly from -10°C to -2°C. The center temperatures of frozen tofu also increased rapidly from 0°C to 10°C in the last 20 min. With the increase

of voltage, the increase rate of the center temperatures raised. As electrode distance increased, the increase rate of the center temperatures decreased. The center temperatures

increased slowly between -2°C and 0°C in all experimental conditions. Most of the thawing time is longer in the temperature range (-2°C – 0°C) than that of other temperature range. The center temperature range (-5°C to -1°C) is often taken as the zone of maximum ice crystal formation in the food freezing industry [14]. When the center temperature of frozen tofu is from -2°C to 0°C , high-voltage electric field treatment exerts its maximum effect. This result coincides with other studies [5]. There is a considerable variation about the thawing time exposed to HVEF at -2°C – 0°C when the voltages, the configuration of the electrodes and electrode distances changed. The thawing time under AC electric field is higher than that under DC electric field. There have different thawing time for the different configuration of the electrodes. The thawing time with needles-to-plate electrodes is faster than that with the other configuration of the electrodes. The thawing time with wires-to-plate electrodes is higher than that with plate-to-plate electrodes. Thus, the electric parameters have a major effect on the thawing time when center temperatures of frozen tofu are from -2°C to 0°C . As can be seen from Figure 3, the results indicate that the rising rate of center temperature increased with rise in voltage from -2°C to 10°C . When the corona wind blows to the surface of the material, the electrical conductivity of the frozen tofu would changes. Then, this would affect HVEF-assisted thawing process. The change of electrical conductivity of samples starts to apply HVEF. However, the effect on thawing is relatively small in the initial stage. The thawing effect increased with rise in the change of electrical conductivity of samples. When the electrical conductivity rises above a certain value, the thawing effect is more remarkable. And there is a great correlation between high voltage and the change of electrical conductivity of samples.

3.3. Evaporation Loss (EL), Thawing Loss (TL), and Drip Loss (DL) Analysis. Evaporation loss, thawing loss, and drip loss of the tofu samples were measured with different thawing parameters in our study. Effect of thawing parameters on the evaporation loss, thawing loss, and drip loss of tofu was given in Figure 4. Water holding capacity has a great relevance with thawing loss. Water holding capacity is high when thawing loss of the material samples is low [2]. The results showed that with the increase of voltages, evaporation loss increased. The evaporation rate of material samples treated with high-voltage electric field significantly accelerated compared to that of the control when the voltage is higher than a specific value [18–20]. Drip loss and thawing loss of frozen tofu were very close under DC electric field. Drip loss and thawing loss treated with high-voltage electric field were less than that with the control. The thawing loss under AC electric field is higher than that under DC electric field. Using the different configuration of the electrodes, the thawing rate is highest with needles-to-plate electrodes and is lower with plate-to-plate electrodes than that under the other experimental conditions. The evaporation loss decreased with increasing applied electrode distance. But drip loss and thawing loss increased with increasing applied

electrode distance. As can be seen, the results indicate that changes in voltage make no great difference to thawing loss under DC electric field. The configuration of the electrodes and electrode distances has significant effects on thawing loss. In other words, water holding capacity of tofu is improved at certain experimental conditions using HVEF thawing. The thawing process can cause the structural changes of the tofu, and this change may lead to an increase in textural properties such as hardness, springiness, cohesiveness, and gumminess, which may match with consumer preferences for harder and springier tofu [21]. In the thawing process, the thawing time has a major effect on thawing loss. The thawing time of frozen tofu was significantly shortened under high-voltage electric field than that of the control.

4. Conclusion

Thawing under HVEF treatment significantly improved the thawing rate and shortened the thawing time of frozen tofu. As the voltage was enhanced, thawing rate increased. Voltage has a little effect on thawing loss under DC electric field. But the configuration of the electrodes and electrode distances has a major effect on thawing loss. In other words, the quality of thawed samples was improved by the HVEF thawing technology. We hope that this study can promote the industrial application of high-voltage electric field in the thawing field and attract more studies about HVEF thawing technique.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

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Research Article

Inactivation of Microbial Food Contamination of Plastic Cups Using Nonthermal Plasma and Hydrogen Peroxide

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The inactivation effect of the combination of nonthermal plasma and hydrogen peroxide aerosol for the microbial decontamination of inner surface of cylindrical container buckets is studied on one bacterial and seven filamentous micromycete species and on airborne-contaminated cups. While the decontamination by single nonthermal plasma or hydrogen peroxide is not observable after 120 s, the strong decontamination by their combination occurs after 30 s of exposure. Moreover, observed total elimination of airborne contamination of plastic cups predetermines this method as a suitable alternative to the currently used method based on the application of hydrogen peroxide.

1. Introduction

The microbial contaminants present a serious problem, among others, in food processing, and their elimination is often desired in many areas. There are many papers studying the microbial decontamination of food and food packaging materials by conventional as well as alternative physical or chemical methods, for example, reviews [1, 2] or research articles as [3, 4] comprise the ultrahigh pressure processing (cold pasteurization), ionizing radiation, electron beam, UV radiation, pulsed electric field, magnetic field, ozone, or other chemical agents as peracetic acid, acetic acid, hydrogen peroxide, linalool, carvacrol, or thymol. Ultrasound is used in [5], pulsed light systems are used in [6] or [7], the effect of radio frequency heating is used in [8], and recently also the shaped electrical pulses have been studied [9].

One possible alternative presents the microbial decontamination by nonthermal plasma (NTP). There are already a lot of works describing its biological effects, devoted mainly to the killing of bacteria or various applications in medicine. See, for example, reviews [10, 11] or other studies [12–18]. The contamination may often arise also from the packaging materials [19, 20]; therefore, many papers describe basic studies of NTP application in food packaging. Several papers deal with decontamination of food packaging materials by NTP generated under low pressure as [21–25]. Following works use NTP in more economically and easier-to-use form at atmospheric pressure, for example, the decontamination of polymer foils [26], plastic tray, aluminum foil and paper cup [27], PET film [28], dried laver [29], shell eggs [30], or sealed packages [31]. Also other effects of NTP treatment on food packaging materials as

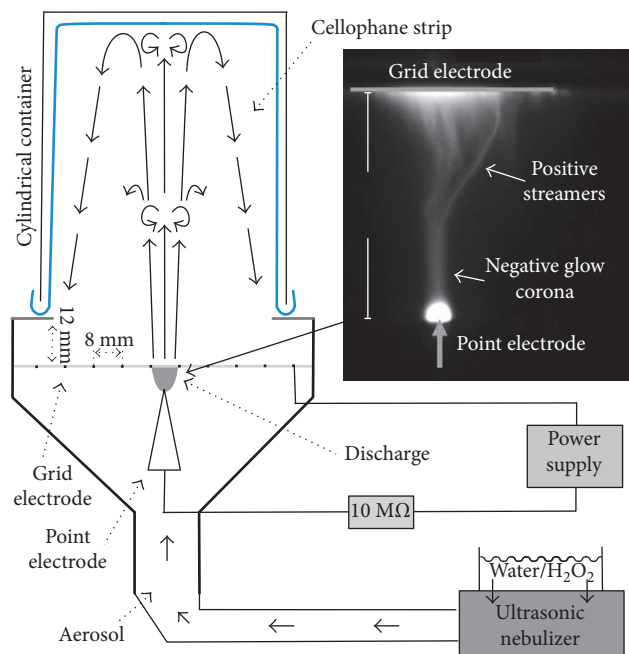


FIGURE 1: Schematic view of the apparatus and picture of plasma active area. Nebulized hydrogen peroxide mixed with air flows to the discharge active plasma region where active particles are produced and carried by the ion wind to the sample.

influence on contact angle, wettability, roughness, and surface energy are studied and summarized in [32, 33].

To increase the decontamination efficiency, the atmosphere where the plasma is generated may be also enriched by some other microbicidal substances, for example, hydrogen peroxide or peracetic acid. Such commercial sterilizers already exist; however, they work at reduced pressure and massive apparatus, resembling autoclave, is needed; for details, see, for example, the review [11] or [34].

In this paper, we combine the benefits of NTP generated at atmospheric pressure, where no additional vacuum apparatus is necessary, and the addition of other bactericidal agent—hydrogen peroxide. This paper follows our previous articles [35, 36], where synergy decontaminating effect of the combination of NTP with hydrogen peroxide aerosol was demonstrated on agar surfaces and dry cellophane foils. Here, we present the study of volume decontamination effect of plastic objects on both defined and airborne contamination. As first, we confirm the previously observed synergy effect in volume also. Following, we have studied the decontamination of large volumes with high concentration of microorganisms so that we could subsequently demonstrate the reliable inactivation of much lower airborne contamination of packaging vessels.

2. Materials and Methods

2.1. Plasma Generation. The NTP was generated in the apparatus previously described in [35, 36]. The point-to-wire discharge burns between the point and grid electrode in an open chamber where the hydrogen peroxide or pure water aerosol was added to the air atmosphere. The point electrode

was represented by the tip of a syringe needle Medoject (0.6×30 mm) situated vertically to the grid electrode. The grid consisted of stainless steel wire of 0.25 mm diameter forming the net with a mesh size of 8 mm. The electrode distance was adjusted to 6 mm. The discharge was stabilized by the connection of a serial resistance of 10 MΩ into the circuit. The polarity of the point electrode was set as negative and the grid electrode as positive. The discharge voltage was set to 2.7 kV which corresponds to the current of 500 μA. For more details about this discharge denominated as pulseless glow, see our previous paper [37], where several stabilized discharges are studied. For other information about the corona discharge stabilization and its characteristic, see, for example, papers [38, 39]. The aerosol of pure water or 10% hydrogen peroxide was generated by the ultrasonic nebulizer (Lucky Reptile, Super Fog SF-1) and mixed with the air; the volume of nebulized water or hydrogen peroxide in the air mixture was 0.40 ± 0.03 ml/l (determined by the weight loss). This mixture flowed to the discharge area and created the discharge atmosphere. The aerosol flow was adjusted to the value of 2.0 ± 0.1 l/min (determined by ebulliometry). The distance between the decontaminated object and the grid electrode was set to 12 mm. The schematic experimental arrangement and the picture of plasma active area are shown in Figure 1.

2.2. Microorganisms under Study. The microorganisms under study were bacteria *Staphylococcus epidermidis* (wild strain) and filamentous micromycetes *Aspergillus oryzae* (DBM 4002), *Aspergillus niger* (DBM 4054), *Cladosporium sphaerospermum* (DBM 4282), *Alternaria* sp. (DBM 4004), *Eurotium* sp. (wild strain 1), *Eurotium* sp. (wild strain 2), and *Trichoderma atroviride* (wild strain). DBM is the Collection of Yeasts and Industrial Microorganisms of the Department of Biochemistry and Microbiology at the University of Chemistry and Technology, Prague. Wild strains were contaminants isolated from food products. In all cases, the bacteria were cultivated on the Mueller-Hinton agar (Oxoid) at 37°C overnight for 18 hours and the micromycetes were cultivated on the Sabouraud medium (Oxoid) agar at 25°C for 5 days to become sporulated.

The suspension of each microorganism under study was prepared by the mixing of loopful taken from the surface of grown culture into the sterile water; in the case of micromycetes, Tween 80 in ratio 0.1% was added into the suspension to improve wettability. It was determined by microscopic examination that the number of conidiospores in the suspension significantly exceeds the number of other mycelium cells.

2.3. Decontamination of Container Buckets. The volume decontamination effect was examined on cylindrical polytetrafluoroethylene (PTFE) container buckets of heights of 13 cm and diameters of 7 cm and 10 cm for volumes of 0.5 l and 1 l, respectively. The inside of containers was lined with contaminated cellophane foils which simplify the microbial manipulation. Prepared suspension of microorganisms was spread homogeneously onto the surface of cellophane foil in

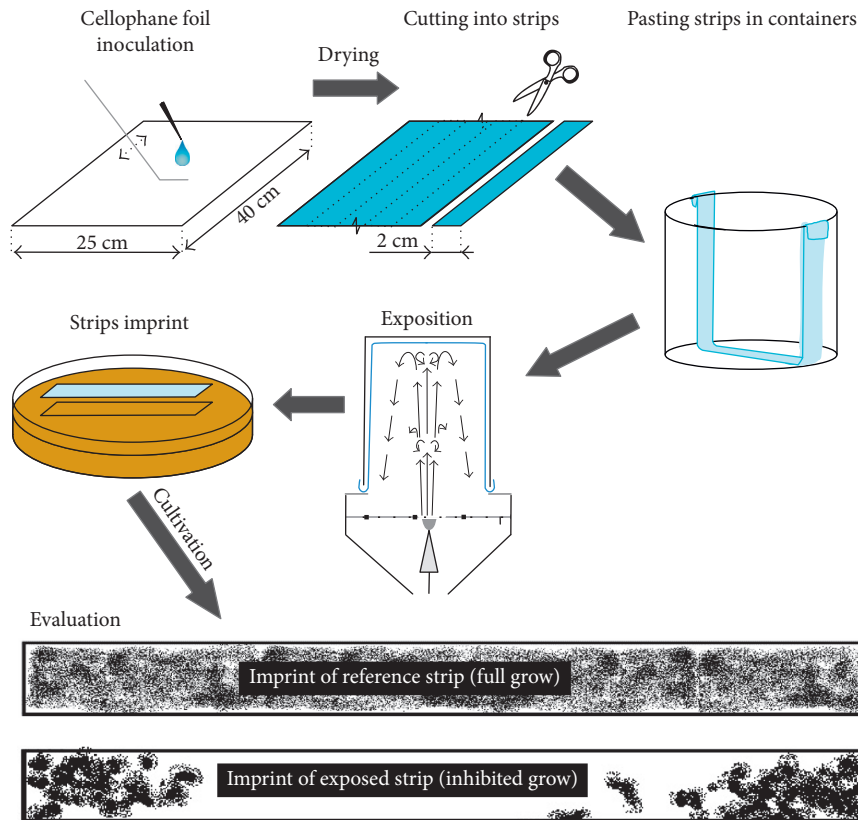


FIGURE 2: Systematics of the experiment. Cellophane foil is inoculated by the microbial suspension, dried, cut into the strips, and placed on the inner surface of cylindrical containers. Containers are exposed to the NTP with hydrogen peroxide aerosol, strips are imprinted onto the surface of the agar medium and cultivated, and the inhibition zones are determined.

the concentrations of 10^4 cfu/cm² and 10^3 cfu/cm² for bacteria and micromycetes, respectively. After one hour drying at common room temperature (22°C) and humidity (40%), the foil was cut into strips (2 cm × 33 cm for 0.51 cup and 2 cm × 36 cm for 1 l cups) and placed on the inner surface of cylindrical containers and exposed to one of described decontamination methods. To transfer the spores from foils to agar, both the exposed and reference foils were imprinted onto the surface of agar and incubated at 37°C for 1 day or at 25°C for 5 days in the case of bacteria or micromycetes, respectively. The operating sequence together with the particular sizes of used foils and cylinders is depicted in Figure 2. All experiments were performed in triplicate.

In first set of exposures, our previous study 36 was followed to confirm the inactivation properties of NTP for 3D objects. Moreover, also the spatial distribution of inhibition effect efficiency was determined. The prepared samples of 0.51 containers with limited number or microorganism species *S. epidermidis*, *C. sphaerospermum*, and *A. niger* were exposed to NTP with addition of pure water aerosol, to NTP with addition of hydrogen peroxide aerosol, and to the hydrogen peroxide aerosol without the NTP. Samples were exposed for 5, 10, 15, 30, 60, and 120 s. The inhibition effect was denoted as full for no colony growth, as partial for observable isolated colonies (lower than approx. 30 cfu/cm²), and the continuous overgrown (higher than approx. 30 cfu/cm²) was denoted as no inhibition.

Afterwards, the efficiency of inactivation for a broader spectrum of all mentioned microorganisms was examined in both container types. Prepared samples were exposed for 30, 60, and 120 s to the combination of NTP with hydrogen peroxide aerosol. The inhibition effect was observed both as the inhibition zone without any growth of colony and as the number of grown cfu.

2.4. Decontamination of Commercial Cups. To confirm the decontamination effect of used apparatus for direct practical application, the decontamination was studied on commercial polypropylene (PP) 0.5 l cups (Wimex s.r. o., Czech Republic) of height of 13.5 cm and of bottom and top diameter of 5.5 cm and 9 cm, respectively. Twenty clean cups were contaminated by artificial or airborne contamination.

For artificial contamination, the inside surface of a cup was sprayed homogeneously by 2 ml of water suspension of *A. oryzae* or *Alternaria* sp. in concentration of 10^3 cfu/ml with Tween 80 in ratio 0.1% and dried for one hour at common room temperature (22°C) and humidity (40%). Consequently, cups were exposed for 10, 15, 30, 60, and 120 s to the combination of NTP with hydrogen peroxide aerosol.

For airborne contamination, cups were kept open in the laboratory for one week and ten of them were exposed to the NTP with hydrogen peroxide aerosol for 30 s.

TABLE 1: Decontamination effect of all used methods in 0.5l cylindrical containers for *S. epidermidis*, *C. sphaerospermum*, and *A. niger* indicated only qualitatively.

Method	Microorganism	Extent of decontamination after exposure time (s)						
		0	5	10	15	30	60	120
NTP with hydrogen peroxide aerosol	<i>S. epidermidis</i>	No	Full	Full	Full	Full	Full	Full
	<i>A. niger</i>	No	No	Partial	Full	Full	Full	Full
	<i>C. sphaerospermum</i>	No	No	Partial	Partial	Full	Full	Full
NTP with pure water aerosol	<i>S. epidermidis</i>	No	No	No	No	No	No	No
	<i>A. niger</i>	No	No	No	No	No	No	No
	<i>C. sphaerospermum</i>	No	No	No	No	No	No	No
Hydrogen peroxide aerosol	<i>S. epidermidis</i>	No	No	No	No	No	No	No
	<i>A. niger</i>	No	No	No	No	No	No	No
	<i>C. sphaerospermum</i>	No	No	No	No	No	No	No

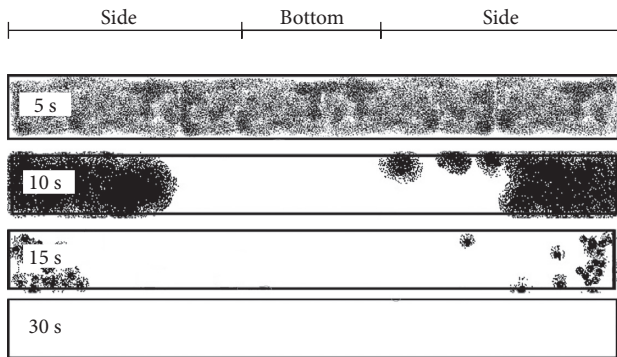


FIGURE 3: The spatial distribution of inhibition effect for *C. sphaerospermum* for several exposure times.

After, all cups were filled with prepared potato-carrot broth with the addition of 50 g of glucose per 1l as model content, closed to prevent contamination, and kept in room temperature for one week. Finally, the number of grown micromycetal colonies on the broth surface was counted. All experiments were performed in triplicate.

3. Results and Discussion

Table 1 shows the results for decontamination of samples contaminated by *S. epidermidis*, *C. sphaerospermum*, and *A. niger* in 0.5l cylindrical container buckets by all three mentioned methods. Obtained results were identical for all three particular repetitions. Observable decontamination occurred after the exposure to the NTP with hydrogen peroxide aerosol only, where the full decontamination occurred within 5 s of exposure for *S. epidermidis* (4 log₁₀ reduction) and within 30 s of exposure for *C. sphaerospermum* and *A. niger* (3 log₁₀ reduction). For other methods, the decontamination was not observable, so that it cannot be declared neither as inhibition zone nor countable colonies even after 120 s. These results correspond with our previous observation and confirm that the combination of NTP and hydrogen peroxide aerosol is much more efficient than their single action for volume decontamination also.

On this basis, in following experiments, the samples were exposed to the NTP in the aerosol of hydrogen peroxide only. The spatial distribution of inhibition effect for *C. sphaerospermum* is depicted in Figure 3. The inhibition

TABLE 2: Decontamination effect of NTP with hydrogen peroxide aerosol in 0.5l cylindrical containers indicated as both inhibition zone (% of area) and total cfu for exposure time (*t*).

Microorganism	Inhibition zone (% of area)/grown cfu		
	<i>t</i> = 30 s	<i>t</i> = 60 s	<i>t</i> = 120 s
<i>S. epidermidis</i>	100/0	100/0	100/0
<i>A. niger</i>	100/0	100/0	100/0
<i>C. sphaerospermum</i>	100/0	100/0	100/0
<i>Eurotium</i> sp. strain 1	100/0	100/0	100/0
<i>Eurotium</i> sp. strain 2	100/0	100/0	100/0
<i>A. oryzae</i>	70 ± 5/10 ± 5	100/0	100/0
<i>T. atroviride</i>	75 ± 5/10 ± 5	100/0	100/0
<i>Alternaria</i> sp.	60 ± 5/30 ± 5	80 ± 5/5 ± 3	100/0

TABLE 3: Decontamination effect of NTP with hydrogen peroxide aerosol in 1l cylindrical containers indicated as both inhibition zone (% of area) and total cfu for exposure time (*t*).

Microorganism	Inhibition zone (% of area)/grown cfu		
	<i>t</i> = 30 s	<i>t</i> = 60 s	<i>t</i> = 120 s
<i>S. epidermidis</i>	100/0	100/0	100/0
<i>A. niger</i>	100/0	100/0	100/0
<i>C. sphaerospermum</i>	100/0	100/0	100/0
<i>Eurotium</i> sp. strain 1	40 ± 5/30 ± 5	100/0	100/0
<i>Eurotium</i> sp. strain 2	35 ± 5/100 ± 10	100/0	100/0
<i>A. oryzae</i>	30 ± 5/10 ± 5	70 ± 5/5 ± 3	100/0
<i>T. atroviride</i>	20 ± 5/10 ± 5	80 ± 5/5 ± 3	100/0
<i>Alternaria</i> sp.	35 ± 5/uncount	75 ± 5/20 ± 5	80 ± 5/5 ± 3

zone becomes to be visible after 10 s exposure in the center which corresponds to the bottom of buckets, and for longer exposure times, it enlarges and takes the whole area. The gradual extending of inhibition zone from the center corresponds with the airflow induced by the discharge ion wind, which is a characteristic of point-to-point/wire/plane discharges. For more details about the plasma sources and their properties, see, for example, our previous works [40, 41] dealing with point-to-point cometary discharge, demonstrative work about the ion source induced airflow [42], or the review of several common plasma sources [43] suitable for decontamination.

TABLE 4: Decontamination effect of all used methods in 0.5l cylindrical containers for *A. oryzae* and *Alternaria* sp.

Microorganism	Grown cfu					
	$t = 0$ s	$t = 10$ s	$t = 15$ s	$t = 30$ s	$t = 60$ s	$t = 120$ s
<i>A. oryzae</i>	Uncount	5	0	0	0	0
<i>Alternaria</i> sp.	Uncount	Uncount	500 ± 100	50 ± 10	4 ± 3	0

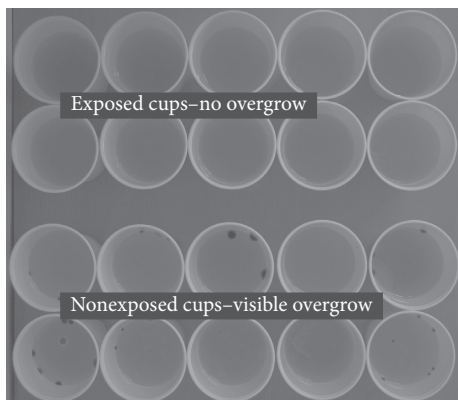
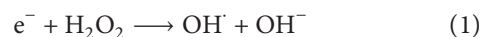


FIGURE 4: The picture of exposed (upper ten) and nonexposed (bottom ten) airborne-contaminated cups after cultivation.

The results of decontamination of all species for both sizes of containers are presented in Tables 2 and 3. In 0.5l containers, the full decontamination occurs for most of species after shortest exposure of 30 s and the full decontamination for all species occurs after exposure of 120 s. In 11 containers, the decontamination after 30 s was only partial for most of species and the total decontamination occurred after 120 s of exposure for all species except *Alternaria* sp. The results show that the bacterium *S. epidermidis* is more sensitive than all micromycetes species and that from selected micromycetal species, *Alternaria* sp. is the most resistant one. As expected, the longer exposure times are necessary for larger volume buckets.

The results for decontamination of artificially contaminated commercial PP cups are given in Table 4. The number of cultivable cfu decreases with the time of exposure. For *A. oryzae*, representing the sensitive species, the strong inhibition occurs after 10 s of exposure and the full inhibition occurs for 15 s and longer exposures. For the most resistant one, *Alternaria* sp., the inhibition effect is visible after 15 s of exposure and the full inhibition occurs after 120 s exposure. For the airborne contamination, it was found that for nonexposed cups, approximately three grown micromycete colonies per one cup are observed (2.5 ± 2.2); however, in exposed cups, no grown colonies are observed. These results make this synergy effect of hydrogen peroxide and nonthermal plasma potentially applicable to the practice. For example, the jam or ketchup production companies in Czechia have permanent problems with spoilage in plastic-packed products. The picture of cups after cultivation is shown in Figure 4. In this case, the number of cfu does not represent the real number of culturable cfu in cups, but it has to demonstrate the potential of present microorganisms to spoil the content, Q.E.D.

In our previously mentioned work [37], the discharge denominated as pulseless glow almost identical with that one used in this study. The discharge burned under similar geometry and identical electrical and chemical characteristics: in time constant voltage of 2.7 kV and current of $500 \mu\text{A}$ where no pulses were recorded by 150 MHz bandwidth oscilloscope; emission spectrum was identical with pulseless glow one. The dominant nitrogen N_2 bands were detected; the lower intensity of N_2^+ indicates the high electron temperature important for microbicidal activity [44, 45]; also the minute peak attributed to OH^\cdot was detected. Fact that the presence of hydrogen peroxide aerosol in the discharge atmosphere did not change its evaluated characteristics indicates that the rapid increase of microbicidal activity must be induced in the postdischarge dark phase. The main mechanisms of hydrogen peroxide activity may be its dissociation (for details, see our previous paper [35]):



Presented apparatus seems to be a potential alternative to several common decontamination methods based on hydrogen peroxide. Addition of the NTP part to current peroxide-based methods would be very easy and cheap and may help to increase the decontamination efficiency, increase the speed of production line, or decrease the amount or concentration of applied peroxide, or solve the contamination problems in production lines where it is difficult to ensure fully aseptic conditions. The decrease of concentration of hydrogen peroxide or amount of energy is also important from an ecological point of view.

4. Conclusions

Experimental study of the volume decontamination effects of nonthermal plasma generated in corona discharge in combination with hydrogen peroxide aerosol shows much higher efficiency than the single action of NTP or hydrogen peroxide. While the decontamination of single nonthermal plasma or hydrogen peroxide is not observable after 120 s, the strong decontamination effect of their combination occurs after 30 s of exposure. This phenomenon was demonstrated on one bacterial and seven micromycete species in both container buckets and 0.5l commercial cups. Along with the total inactivation of airborne contamination of commercial cups, it pre-determines this method as a suitable alternative to the currently used methods based on hydrogen peroxide applications. It may help to reduce the required amount or concentration of hydrogen peroxide, which is also important from an ecological point of view.

Data Availability

In Tables 1–4, only the mean values and standard deviations are presented. The reader can access the particular results on request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Review Article

Three Pillars of Novel Nonthermal Food Technologies: Food Safety, Quality, and Environment

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This review gives an overview of the impact of novel nonthermal food technologies on food safety, on quality, and on the environment. It confirms that research in this field is mainly focused on analyzing microbial and/or chemical aspects of food safety. However, recent research shows that in spite of various food safety benefits, some negative (quality oriented) features occur. Finally, this paper shows the necessity of analyzing the environmental dimension of using these technologies.

1. Introduction

Nonthermal technologies are used in interdisciplinary sciences, in biotechnology, and in many other research and applied areas. In food processing, they are used mainly for preservation in treating of food and wastewaters. Consumer demands for minimally processed foods in addition to the negative effect of heat on nutritional properties of foods are making nonthermal processing popular in the food industry. The main task of nonthermal processing is to assure food safety [1], and research effort is focused on microbial inactivation, food safety, and preservation while retaining the quality of obtained products. This advantage gives nonthermal processing the potential to replace classical thermal processing. Besides the food safety and quality dimensions, these processing technologies have the possibility to shorten treatment time, lower energy consumption, and lower carbon footprint [2].

Nonthermal technologies have different types of action, depending on the source of energy transfer. They are used in inactivation of microorganisms in radical formation (plasma,

ultrasound, ozonation, UV light, etc.); mechanical action through hydrodynamic effects, shock waves (ultrasound and plasma), electric and magnetic fields (pulsed electric fields, cold plasma, radiofrequency and oscillating magnetic fields, electrohydrodynamic processing, and electron beam processing); or extremely high pressures that are causing rupturing and bursting of microorganisms [3–6]. These treatments may be used alone or in combination, within the so-called “hurdle” concept [7–9]. The most researched techniques with proven scientific results in the food industry are high-pressure processing (HPP), supercritical fluid extraction (scCO₂), and pulsed electric fields (PEFs).

There are many research projects dealing with microbial inactivation [10–12], enzyme inactivation [11, 13], and nutritional improvements [14–16] when using nonthermal technologies. All of these techniques have been successfully applied in assuring food safety [17–20]. However, besides assuring food safety, more attention is needed to maintain or improve food quality. Quality of food after nonthermal processing has shown both positive [21, 22] and negative [23–25] effects depending on the technique and processing

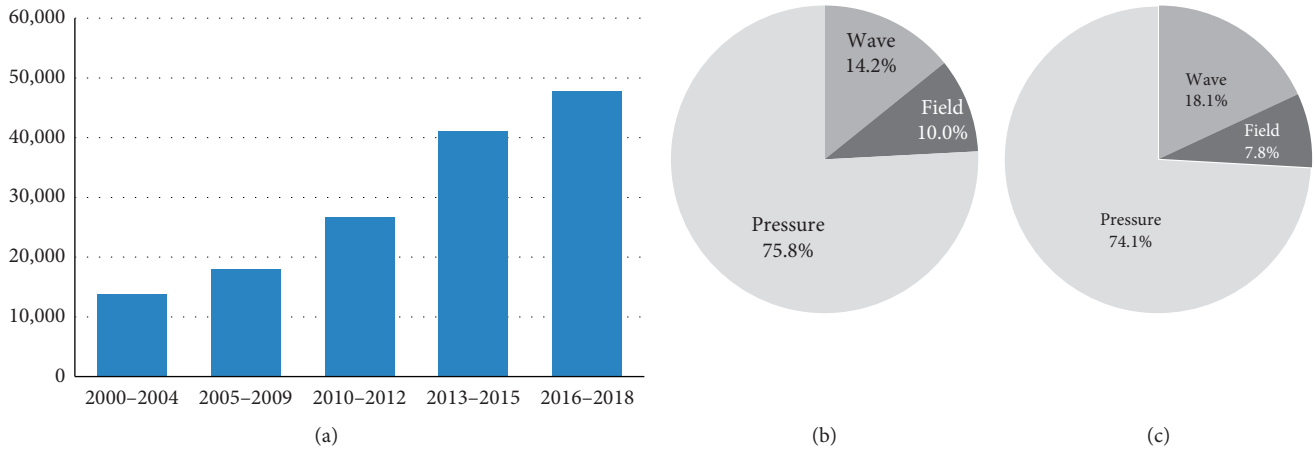


FIGURE 1: Presentation of publications related to the application of nonthermal technologies in the food industry: (a) number of publications; (b) share of publications; (c) share of patents. Wave: ultraviolet, ultrasound, and plasma; field: electric and magnetic fields' technologies; pressure: high-pressure processing/homogenization.

parameters. This raises the first challenge in succeeding inactivation of microorganisms while impairing quality and sensory parameters of treated samples and opens a research gap of unresearched areas like negative aspects of application of novel nonthermal processing on food quality, stability of food during shelf life after nonthermal processing, negative sensory properties of food treated by novel nonthermal techniques, life cycle assessment, and sustainability of nonthermal processing techniques. Advantages of novel nonthermal processing in terms of energy consumption can be considered as “green” techniques for “green” extraction. In order to gain better output products using nonthermal processing, it is important to overview processing in terms of safety, quality, and environmental aspects.

The objective of this review paper was to present the three main pillars related to the use of novel food technologies—food safety, quality, and environmental impacts on the one side stressing advantages and constraints and on the other side revealing future synergic research perspectives.

2. Materials and Methods

Online literature on the use of nonthermal technologies in the food industry is dispersed in a heterogeneous way in the form of scientific manuscripts, book chapters, conference proceedings as well as patents, legislation, and even company reports. According to our goal, we carried out a search in scientific literature spanning the research for the period 2000–2018. The authors mainly focused the attention on the international journals to assure a more scientific content mainly caused by a rigorous revision process. Therefore, the selection of scientific manuscripts was based on the journals impact factor, matching to the scope of the journal and preferring those indexed by international repositories such as the Scopus index and publishers (Elsevier, Springer, Wiley, Taylor and Francis, and EBSCO). This research identified relevant articles, both review and research papers, published in the domains of nonthermal technologies split

into two subsections: specific nonthermal technologies (HPP, scCO₂, PEF, etc.) and its application on the specific type of food (beverages, fruit, vegetables, etc.). There were no geographical restrictions applied.

This type of literature review identified that there are over 300,000 publications related to the application of nonthermal technology in the food industry. In this millennium, the number is increasing as presented in Figure 1(a) where the period before 2010 was divided into two five-year periods: 2000–2004 and 2005–2009. The period starting from 2010 was analysed in three-year periods. Although there are papers published in journals that are not strictly in the “food science and technology” scope, the top five journals that have at least 500 publications are Food Chemistry, LWT Food Science and Technology, Journal of Food Engineering, Innovative Food Science and Emerging Technologies, and Food Research International. The journal covering the environmental impact of these technologies is published in the Journal of Cleaner Production and Bioresource Technology. Depending on the type of technology, the share of publications and patents was analysed and is presented in Figures 1(b) and 1(c). It is important to note that high-pressure processing and homogenization are the most analysed technologies in around 75% of all research/review publications. The same applies to publication of patents.

Deeper analysis of patents reveals that the majority of patents were published in journals up to 2010 and covered patents of new nonthermal technologies, food substitution with novel food derived from new technologies, and aspects of food preservation using these technologies. Majority of patents came from the developed countries (EU, USA, China, Japan, Australia, etc.), and no other patterns were observed.

A literature review revealed that these technologies were evaluated separately either from a food technology/food safety perspective or from an environmental perspective. Combination of two types of criteria—environmental and quality/food safety—has not been a focus of research, and

this has been identified as a research gap by the authors of this paper.

3. Safety of Food Processed with Nonthermal Technologies

Nonthermally processed food presents some kind of a risk due to incomplete preservation of food. At the beginning of an extensive research and application of nonthermal food technologies, the US Food and Drug Administration (FDA) requested the Institute of Food Technologists (IFT) to give a report on the effectiveness of microbial inactivation of alternative food-processing technologies. Back in 2000, the IFT reported general guidance for future research on novel techniques based on microbiological demands like the evaluation of the adequate linear first-order survivor curve model and launching experimental protocol, identifying inactivation action/mechanism(s) among alternative technologies, and determining the synergism or antagonism of one alternative processes [26]. The IFT also emphasized the importance to determine potential formation of indigestible and toxic by-products of processing as well to develop methods for measuring and monitoring physical-chemical changes during treatments [26].

As a result, from the year 2004, the definition of pasteurization changed and now, according to the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the USDA, includes any process, treatment, or combination, which is applied to food to assure microbial safety [27]. In order to evaluate alternative pasteurization methods, there are several steps to pay attention on, like the properties and composition of the treated food product, microbial effects, and commercial, economic, and environmental aspects [28]. Each of these technologies has specific critical process parameters that must be monitored and controlled (critical control points).

The Novel Food Regulation by the EU lacks a joint classification of new technologies across all member states which slows down their widespread commercialization in Europe. In order to proceed with the technology readiness levels of novel food technologies, one of the necessary prerequisites is to validate them in relevant environment [29]. Novel food technologies may be used for different purposes in the food industry [30], such as (1) preservation/decontamination and shelf life extension, (2) food modification (i.e., gelatinization), (3) stress induction (i.e., increase in biosynthetic activities), (4) mass transfer modification (i.e., extraction), and so on.

In parallel with developing nonthermal technologies, it is of vital importance that equipment is hygienically designed [31]. Hygienic design is defined as “design and engineering of equipment and premises assuring that food is safe and suitable for human consumption” [32]. It is not widely understood, and there is still little awareness of possible consequences of equipment that is not hygienically designed [33]. Public health and economic aspects of microbial contamination in foods may cause financial and public concerns, particularly if these result in product recalls. The hygienic design of equipment plays an important role not

only in controlling the microbiological safety and quality of the products made but also in prevention of residues of chemicals used for cleaning and disinfection. Also, the hygienic design should prevent food from being contaminated with other contaminants. This becomes more important with novel food-processing technologies where new designs may result in new (re)contamination pathways, while minimized food processing may not achieve heat-standard inactivation [9].

Legislation covering the hygienic design of food-processing equipment is vague [34]. In the EU, some legislation mentions the importance of the hygienic design such as the regulation of hygiene of foodstuffs [35]. On the contrary, there are a number of different types of standards related to the hygienic design with different approaches in highlighting similar hygiene issues [33]. Most used standards that outline requirements related to the hygienic design are industry-tailored guidelines or sanitary standards [36–39].

3.1. Microbial Food Safety. All aspects of microbial food safety need to be monitored, and this includes assuring FDA regulative for 5 log reduction using preservation processes. High-pressure processing (HPP) and pulsed electric fields (PEFs) have been greatly researched and proved to be successful in assuring food safety, and by that, they were successfully commercialized [4, 40, 41] dealing with non-thermal inactivation of microorganisms. PEF provides minimal changes in food attributes while assuring optimum safety. Pulsed light is used in decontamination of various (transparent) liquids. However, limitation of this technology leads to undesirable results, such as decomposition of nutrients and changes in sensory quality. Food safety is one of the important components that force the development of novel technologies to reduce, control, or eliminate food-borne pathogens from food products and contact surfaces. State of the art of assuring and demonstrating 5 log reduction was described in terms of applying optimized nonthermal treatment [1, 20, 42]. Nonthermal techniques can be combined [10, 13, 43–46] or be used with antimicrobial agents [47–49] or in combination with mild heating [1, 22, 50]. It is necessary to follow critical control processes of nonthermal processing [51–53] in order to have no recovery or revitalization of microorganisms after processing [54, 55]. There are laboratories in the United States that validate food process for all nonthermal techniques, and processing needs to be evaluated and overviewed for microbial stability, toxicology, interactions between the product and packaging, chemistry, and so on. Foods derived from these technologies are also subject to this kind of validation.

High pressures result in extreme mixing and high-intensity shear forces moving throughout the medium. This release can result in examples of aggregate disruption, polymer chain fractures, and chain length degradation causing permanent changes in molecules [56]. This mechanism works particularly well at low frequencies such as 20 and 40 kHz. Although ultrasound has shown some

benefits to processing of foods, for example, in extraction, crystallization, and microbial inactivation, there are also some concerns as expressed by Pingret et al. [25] who presented a review on the possible degradative effects of sonication on food with high lipid contents and indicated the possible degradation of some compounds and changes to physiochemical qualities of some food products.

The extent of microbial inactivation by HPP in foods depends on multiple factors related to the processing conditions and the food matrix. Additionally, it was broadly observed that different microorganisms express a wide range of sensitivity to HPP [57, 58].

In particular, prokaryotic cells are observed to be more pressure resistant than eukaryotes [57], yeasts and moulds are relatively more HPP sensitive than bacteria, and Gram-positive bacteria are more resistant to pressure than Gram-negative bacteria, likely because the higher complexity of the cell or of the cell membrane might increase HPP susceptibility [59]. Additionally, cocci are more resistant than rod-shaped bacteria [59].

However, the most resistant species are the endospores, which are capable of withstanding pressures $>1,000$ MPa [59]. HPP is reported to induce the germination of bacterial spores, at an extent depending on the food matrix and the microorganism [59, 60].

In general, the efficiency of high-pressure homogenization (HPH) for microbial inactivation depends on the properties of the process fluid (viscosity, temperature, suspended solids, or fats), the specific resistance of the microbial strains, and the operating conditions, such as the operating pressure, the number of HPH passes, the operating temperature, and the homogenizing valve geometry [61]. The pressure limit separating HPH (high-pressure homogenization) from UHPH (ultrahigh-pressure homogenization) is not clearly defined, whereas pressures above 200 MPa are often named UHPH [62, 63].

The inactivation kinetics for most microorganisms appear to be first order with respect to the applied pressure, in the range of HPH and UHPH pressure levels (100–350 MPa) [64]. In contrast, for repeated HPH passes, an asymptotic behavior is generally observed, which can be attributed to the natural distribution of individual cell resistance to pressure [65]. Moreover, the homogenizing valve geometry also appears to be determining factors for microbial inactivation. In fact, the microbial inactivation is a direct consequence of the physical cell disruption due to the fluid-mechanical stresses generated in the valve, such as shear and elongational stresses, turbulence, cavitation, and impact on the valve surfaces, which depend on the specific valve design [66, 67].

Due to the temperature rise in the homogenization valve and due to the frictional heating associated with the pressure energy dissipation, the thermal inactivation of the microorganisms is likely to occur during HPH treatments, if the inlet and outlet temperatures are not carefully controlled.

If a purely nonthermal treatment is desired, to preserve the thermosensitive food components, the inlet temperature should be adjusted as a function of the operating pressure, taking into account the inherent heating of the system

(generally comprised between 0.15 and 0.22°C/MPa) [68]. Moreover, also a heat exchanger should be placed immediately downstream of the homogenizing valve, which is desirable to minimize thermal damage to the product.

Both HPH and UHPH treatments primarily kill the vegetative bacteria, through the mechanical destruction of the cell integrity [61, 67]. Gram-positive bacteria are reported to be more resistant than Gram-negative bacteria, which have thinner cell walls, formed by 1–5 layers of peptidoglycan chains, in comparison with the 40 layers of peptidoglycan chains of the Gram-positive bacteria [69, 70].

Yeasts and fungi exhibit an HPH resistance, which is intermediate between Gram-negative and Gram-positive bacteria, because of their wall structure, which is thicker than that in Gram-positive bacteria, but more complex than that in Gram-positive bacteria due to the larger size and a different cell wall structure, with glucans, mannans, and proteins as basic structural components [71].

There are different actions of nonthermal processing, but in some use of one technology *per se* is not enough to assure inactivation of microorganisms in a significant way. Efficiency in inactivation of microorganisms by those treatments differs depending on treatment parameters like treatment time, power, strength, dosage, frequency, and so on. On the other hand, by working at lower temperatures, there are possibilities that applied treatment is not enough in prolonging the shelf life of the product and there are significant hazards like re-vegetation and recovery of microorganisms (sublethal injuries, stress, viable but nonculturable state, etc.). When nonthermal treatments achieve food safety, there is possible deterioration of food quality. One example is treated wine which was treated by ultrasound. Ultrasound caused the formation of negative oxidative smell and the formation of aromas which are described by panellists as burns or smoke [72]. It is explained by the formation of oxidized aroma (acetaldehyde) in young red wines, that is, the reaction of wine polyphenols (initiated by the ultrasound treatment) to form peroxide which oxidizes ethanol to acetaldehyde. This is often observed in high oxidative techniques (plasma, ultrasound, etc.). Through formation of free radical and high reactive oxygen or nitrogen species, the nonthermal processing can be efficiently introduced in wastewater treatments and recovery of agro and food waste.

Another area of safety concerns within food processing is sterilization and reduction of contamination by bacteria and other microbes. Ultrasound has been shown to be very effective in treating the rate of bacterial growth and increasing the kill rate of microbes at a range of frequencies, the most effective being 850 kHz due to the short life span of the cavities at this frequency. It is thought to affect microbial inactivation via the weakening or disruption of bacterial cells through a number of different processes which include mechanical and chemical effects. Mechanical effects are induced by sonication at lower frequencies of 20 kHz, as a result of increased pressure gradients formed during the collapse of cavitation bubbles within or near the bacteria, which result in enhanced shear forces, microstreaming, and high levels of mixing resulting in disruption of the bacteria. Evidence continues to grow for the use of ultrasound in the

TABLE 1: Ultrasonic parameters and effects on various bacteria and fungi.

Author(s)	Type of food	Bacteria and fungi	Experimental setup parameters	Effects of ultrasound treatment
[73]	Skimmed milk	<i>Enterobacter aerogenes</i> , <i>Bacillus subtilis</i> , <i>Staphylococcus epidermidis</i> , <i>S. epidermidis</i> , <i>Staphylococcus pseudintermedius</i>	$\nu = 20$ kHz; $P = 13$ W; $t = 20$ min; $T = 30^\circ\text{C}$	Up to 4.5 log reduction for <i>E. aerogenes</i> and <i>B. subtilis</i> . <i>Staphylococcus</i> spp. not affected
[74]	Skim milk powder	<i>Geobacillus stearothermophilus</i>	$\nu = 20$ kHz, $T = 45^\circ\text{C}$, $t = 30$ s for cells; $\nu = 20$ kHz, $T = 67.5^\circ\text{C}$, $t = 17.5$ s for spores	Cell reduction (4.8 log) at 19.75% total solids and spore reduction (0.45 log) at 31.5% total solids
[75]	Pomegranate juice	<i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i>	$\nu = 20$ kHz; amplitude levels of 50, 75, and 100%; $t = 0, 3, 6, 9, 12, 15$ min; $T = 25 \pm 1^\circ\text{C}$	100% amplitude level for 15 min, reduced levels by 3.47 and 1.86 log-cfu/mL, respectively
[76, 77]	Strawberry, orange, apple, pineapple, and red fruit juice	<i>Saccharomyces cerevisiae</i> , <i>Pichia membranifaciens</i> , <i>Wickerhamomyces anomalus</i> , <i>Zygosaccharomyces bailii</i> , <i>Zygosaccharomyces rouxii</i> , <i>Candida norvegica</i>	$\nu = 20$ kHz; $P = 130$ W; amplitude levels 20% to 60%; pulse = 2, 6 s; $t = 2$ –6 min	Reduction of spoilage organisms
[78]	Cactus pear juice	<i>Escherichia coli</i>	$\nu = 20$ kHz; $P = 1500$ W; amplitude levels of 60%, 70%, 80%, and 90%; $t = 1, 3, 5$ min	Total inactivation in both fruit juices after 5 min of ultrasound treatment at most amplitude levels
[79]	Ayran, an acidic milk drink	<i>Streptococcus thermophilus</i> and <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	$\nu = 35$ kHz; $T = 60, 70, 80^\circ\text{C}$; $t = 1, 3, 5$ min	Counts decreased as the temperature and time increased
[80]	Orange juice	<i>Alicyclobacillus acidoterrestris</i> spores	$\nu = 24$ kHz; AI = 460 W/cm ² ; $P = 33$ W; AI = 105 W/cm ² ; $P = 162$ W	Thermosonication required at least 8°C lower temperatures than thermal treatments to achieve the same spore inactivation
[81, 82]	Natural squeezed apple juices	<i>Alicyclobacillus acidoterrestris</i> spores and <i>Saccharomyces cerevisiae</i>	$\nu = 20$ kHz; $P = 600$ W and 95.2 μm wave amplitude; $t = 10$ or 30 min; $T = 20, 30$, or $44 \pm 1^\circ\text{C}$ and pulsed light (PL) (xenon 1 and 3 pulses/s; 0.1 m distance; 2.4–71.6 J/cm ² ; initial $T = 20, 30, 44 \pm 1^\circ\text{C}$)	Combination of these technologies led up to 3.0 log of spore reduction in commercial apple juice and 2.0 log in natural juice; for <i>S. cerevisiae</i> , 6.4 log reduction and 5.8 log reduction were achieved
[82]	Apple juices	<i>Escherichia coli</i> ATCC 35218, <i>Salmonella Enteritidis</i> MA44 and <i>Saccharomyces cerevisiae</i>	$\nu = 20$ kHz; $P = 600$ W and 95.2 μm wave amplitude; pulsed light 0.73 J/cm ² , 155 mL/min	Combined ultrasound and pulsed light led up to 3.7–6.3 log reductions of inoculated microorganisms
[83]	Milk	<i>Escherichia coli</i> , <i>Pseudomonas fluorescens</i> , <i>Staphylococcus aureus</i> , and <i>Debaryomyces hansenii</i>	$\nu = 24$ kHz; amplitude levels 70 and 100%; $t = 50, 100, 200, 300$ s	Population reduction. Milk deterioration
[75, 84]	Pomegranate juice	<i>Escherichia coli</i> and <i>Saccharomyces cerevisiae</i>	$\nu = 20$ kHz; amplitudes levels 50, 75, and 100%; $t = 0, 6, 12, 18, 24, 30$ min	More than a 5 log inactivation of <i>E. coli</i> and a 1.36 log inactivation of <i>S. cerevisiae</i>
[85]	Orange juice	<i>Saccharomyces cerevisiae</i>	$\nu = 20$ kHz, $P = 778.2$ W ultrasonic power, $t = 11$ min; $P = 350$ W microwave power, $T = 35^\circ\text{C}$	Complete inactivation

TABLE 1: Continued.

Author(s)	Type of food	Bacteria and fungi	Experimental setup parameters	Effects of ultrasound treatment
[86]	Fresh produce	Gram-positive bacterial strain <i>Listeria innocua</i> and Gram-negative bacterial strains <i>Escherichia coli</i> O157:H7 and <i>Pseudomonas fluorescens</i>	$t < 10$ min	0.5 log-CFU/cm ² reduction of all strains when washed with ultrasound
[87]	Chicken	<i>Campylobacter jejuni</i> and <i>spoilage bacteria</i>	$T = 4^{\circ}\text{C}, 25^{\circ}\text{C}, 54^{\circ}\text{C};$ $t = 1, 2, 3$ min	Treatments significantly reduced total viable counts
[88]	Salads	<i>Listeria monocytogenes</i>	$t = 5$ min in water inoculated with herbs/spices	Samples exhibited up to 4 log reduction 1 day after treatment

v, frequency (kHz); T, treatment temperature ($^{\circ}\text{C}$); t, treatment time (min or s); P, treatment power (W); AI, acoustic intensity (W/cm^2).

deactivation and sterilization of many different bacterial strains. This can be achieved within short treatment times with higher frequencies of sonication, thus resulting in minimal disruption to the food material itself. An overview of ultrasound effects on microbial safety is presented in Table 1.

Liu et al. [89] investigated the inactivation of *Saccharomyces cerevisiae* under varying conditions such as bacterial load concentration, pH, and treatment temperature and determined that ultrasound had the most significant role in the inactivation of the bacteria. Kang et al. [90] examined the effects of ultrasound on the number of *Escherichia coli* O157:H7 and vegetative cells of *Bacillus cereus* in brining and beef during the curing processing. After 30 min of treatment, *E. coli* appeared to be more affected by sonication than the *B. cereus*. This was thought to be due to the formation of hydrogen peroxide acting as a sterilization agent as a result of recombination reactions of OH radicals during the sonication process. Sienkiewicz et al. [91] examined the growth of the strain of *Salmonella enterica* subsp. *typhimurium* during sonication. Total inactivation of *Salmonella* spp. occurred with low bacterial populations after sonication at 20 and 40 kHz for 30 min and with high bacterial population at 20 kHz for 30 min with reductions observed after only 15 min of treatment. Bacterial inactivation, after sonication, lasted for up to 48 h in storage at 21°C.

The levels of *Campylobacter jejuni* and spoilage organisms in raw chicken were examined by Kassem et al. [87] who employed sonication alone or in combination with different solutions containing either lactic acid, sodium decanoate, or trisodium phosphate at a range of temperatures and treatment times. While all the solutions exhibited some reduced bacteria levels as compared to the control, combination treatments fared far better with only sonication in conjunction with 3% sodium decanoate solution showing any significant improvements and much reduced total viable counts.

Khandpur and Gogate [48] investigated microbial growth in a range of fruit and vegetable juices via the application of sonication in the presence and absence of crude orange oil and compared these to thermal controls alongside other quality parameters such as pH, acidity, Brix, and yeast content. The optimized ultrasound parameters for juice sterilization were ultrasound frequency and power of 20 kHz and 100 W with a 15 min treatment time, and more than 5 log reduction was achieved with lower microbial growth and improved quality characteristics as compared to the thermally processed juice.

3.2. Chemical Food Safety. Milne et al. [92] examined OH^{*} radical formation employing ultrasonic frequencies. Comeskey et al. [93] also employed a range of ultrasonic frequencies to determine levels of hydrogen peroxide formed in sonicated aqueous systems. Using a range of ultrasonic frequencies, they determined that the highest levels of hydrogen peroxide occurred at 850 kHz with

380 and 512 kHz also exhibiting some oxidative effects however not to the same extent.

Kang et al. [94] investigated treatment time versus ultrasonic power in an attempt to examine the oxidation of beef proteins. They determined that sonicating beef under varying treatment conditions greatly increased the amount of lipid oxidation compared to static brining. Protein oxidation was determined by examination of carbonyl levels and levels of disulphide cross-linking, which indicated a decrease in total sulfhydryl, as a result of free radicals contributing to protein oxidation. Continuing their work, Kang et al. [90, 95] sonicated beef at 150 and 300 W for 30 and 120 min and found that this increased the water holding capacity and tenderness of the beef as compared to salt brining. This was in this case attributed to induced oxidation of myosin causing polymerization of the muscle fibres, thus increasing the water holding capacity of the meat.

Sun et al. [96] examined the link between anthocyanin degradation and ultrasonically formed hydroxyl radicals. They discovered that the absorbance of the antioxidant cyanidin-3-glucosylrutinoside at 282 and 518 nm decreased significantly on increased sonication which was confirmed by 1,1-diphenyl-2-picrylhydrazyl and ferric-reducing antioxidant assays, thus indicating a negative effect on antioxidant levels as a direct result of extended sonication. Yao et al. [97] also observed a similar effect when examining the effect of sonication on antioxidant levels in blueberries and discovered that sonication significantly increases the degradation of cyanidin-3-glucoside as compared to thermal treatments.

The inactivation of horseradish peroxidase was investigated by Tsikrika et al. [98] who determined that sonication for 60 min using 20, 378, 583, 862, 995, 1144, and 1175 Hz ultrasound at power levels (acoustic energy) between 2.1 and 64 W was very effective at inactivating the enzymes with little effect observed at the 20 kHz lower frequency. The fact that the greatest levels of inactivation were observed at 378 and 583 nm suggests that some radical effect may be the cause. There is much evidence presented to suggest that it is the higher frequencies of sonication, above 370 kHz to 850 kHz, which result in high levels of oxidative radical formation. It is therefore suggested that, in order to avoid radical interference with food materials, lower frequencies for treatment should be employed with shorter sonication times to limit any oxidative effects.

Food allergies have posed a severe risk in the last decade. According to [99], allergic reactions are caused mainly due to "epitopes," a small linear stretch of amino acids or a specific three-dimensional structure which is a part of a much larger protein. During food processing (both conventional and novel), the epitopes that are present within the food matrix may be destroyed or new epitopes may be formed. Also, these technologies can result in producing conformational changes in the protein structure and formation of epitope centres, but also few of them can be used for the future development of the hypoallergenic foods by reduction or by mitigation of the reactivity on processing. Scientists are still trying to explain and understand the

conformational changes in the protein which can affect the allergenicity.

4. Quality of Food Processed with Nonthermal Technologies

Stakeholders, such as legislators, retailers, and manufacturers, care about consumers' opinion regarding food processed with novel technologies [100]. Giving the possibility to evaluate and to taste, a novel product seems to influence consumer acceptance for new technologies. This is still largely an unexplored area, but it has been suggested that including consumers in the process of evaluation, that is, by pairing the new technology with a positive sensory experience of the product, can lead to the positive consumers' reaction [101].

High-pressure processing (HPP) might significantly impact the quality and functionality of food, affecting the color, flavor, and texture, with a relevant impact on sensory perception and consumer acceptance [61]. Generally, HPP, by slowing down some biological reactions, such as Maillard's reactions, contributes also indirectly to a better preservation of the quality attributes and nutritional value over their shelf life [102]. In addition, HPP is reported to have only a limited effect on the covalent bonds of low-molecular-mass compounds, such as those responsible for color, flavor, and health-beneficial attributes. For example, HPP at low and moderate temperatures did not cause any significant alterations of the pigments, such as chlorophyll, carotenoids, and anthocyanins, responsible for the color of fruit juices [103]. The color compounds can, however, change during the storage of HPP-treated products more rapidly than in thermally treated ones, due to the incomplete inactivation of enzymes and microorganisms by high pressure [61].

Several studies clearly showed that the flavor of fruit juices is not affected by HPP because the structure of small molecular flavor compounds is only marginally affected by high pressure [104–107]. However, similar to pigments, the flavor of fruits and vegetables subjected to HPP might be indirectly altered, through enhancing or delaying of some enzymatic reactions, which might alter the balance of flavor composition [105, 108, 109]. HPP is also reported to affect the rheological behavior of the juices and their cloud stability, as both these parameters are controlled by the composition of the soluble pectins. Pectin breakdown or retention, induced by HPP also through enzymatic reactions, enables the control of the rheological behavior of the juice. For example, the residual activity of pectin methyl-esterase, not completely inactivated by HPP, caused a decrease in orange juice viscosity during its shelf life [109]. In contrast, the viscosity of tomato juice was observed to increase linearly with pressure, in the high pressure range (200–500 MPa), whereas at low pressures (100–200 MPa), a decrease in viscosity was observed because of enzymatic degradation of pectins [110].

One of the main quality indicators upon food processing is the preservation of the content of bioactive molecules, which might contribute to the health-beneficial properties of

the food products. Remarkably, nonthermal technologies not only are reported to better preserve bioactives than thermal treatments but also in some cases can stimulate their release from the intact cells contained in the product, which translates in the increase of instrumentally detectable bioactive concentration and, often, of their bioaccessibility. In fact, HPP of vegetable cells at 250 MPa for 10 min was reported to induce 99% of the pigment to be released [111].

Figure 2(a) summarizes the effect of HPP on different bioactive compounds, in comparison with the corresponding values in untreated products.

Vitamin A was reported to increase slightly in orange juice, treated between 100 and 400 MPa for 1–5 min at temperatures between 30 and 60°C [112], and significantly in apple juice treated for 5 min at 400 and 500 MPa and 25 and 45°C [61].

In contrast, different authors reported that vitamin C decreased upon high-pressure homogenization (HPH) and ultrahigh-pressure homogenization (UHPH) treatment, as observed in orange juice [112], in blueberry juice treated at HPP pressures between 200 and 600 MPa and at temperatures of 42°C for 5–15 min [113], and in melon pieces treated for 10 min at HPP 600 MPa and ambient temperature [114]. In the case of vitamin E, a slight decrease was observed in rosehip puree, HPP treated at 200–600 MPa and 20°C for 5 or 10 min [115], whereas a measurable increase was reported by the same authors for spinach leaves [115] and in sliced ham, HPP treated at 400–900 MPa and 12°C for 10 min [116].

In the case of total carotenoids and of their main components, such as β -carotene, lutein, and zeaxanthin, it is generally reported that they are well preserved during HPP. In rosehip puree, similar to what reported for vitamin E, a decrease in total carotenoids, and in particular in lutein and zeaxanthin, was observed, while in spinach leaves, the total carotenoids were reported to increase [115].

In melon pieces treated by HPP, β -carotene was observed to increase, differently from what was observed for vitamin C [114]. In orange juice treated between 100 and 400 MPa for 1–5 min at temperatures between 30 and 60°C, β -carotene, lutein, and zeaxanthin significantly increased [112]. Remarkably, in bee pollen paste treated at 200–400 MPa and 20°C for 5–15 min, a substantial increase in total carotenoids was observed [117].

The anthocyanins slightly decreased in blood orange juice treated at 400–600 MPa and 20°C for 15 min [118], while significantly increased in must obtained from grapes treated at 400–550 MPa and 20°C for 10 min [119].

HPP at 400–600 MPa and 25–50°C for 5 or 10 min caused a decrease of the total polyphenols in pomegranate juice [106], whereas in blueberry juice [113] and in bee pollen paste [117], total polyphenols significantly increased. In the preservation of anthocyanins and polyphenols, a significant role is also played by the inactivation by HPP of the enzymes responsible for their degradation [120].

HPH and UHPH treatments are reported to better preserve the natural functional compounds of the juices, such as vitamins C and A, flavonoids, and polyphenols, while reducing the microbial load to the desired value [121–123].

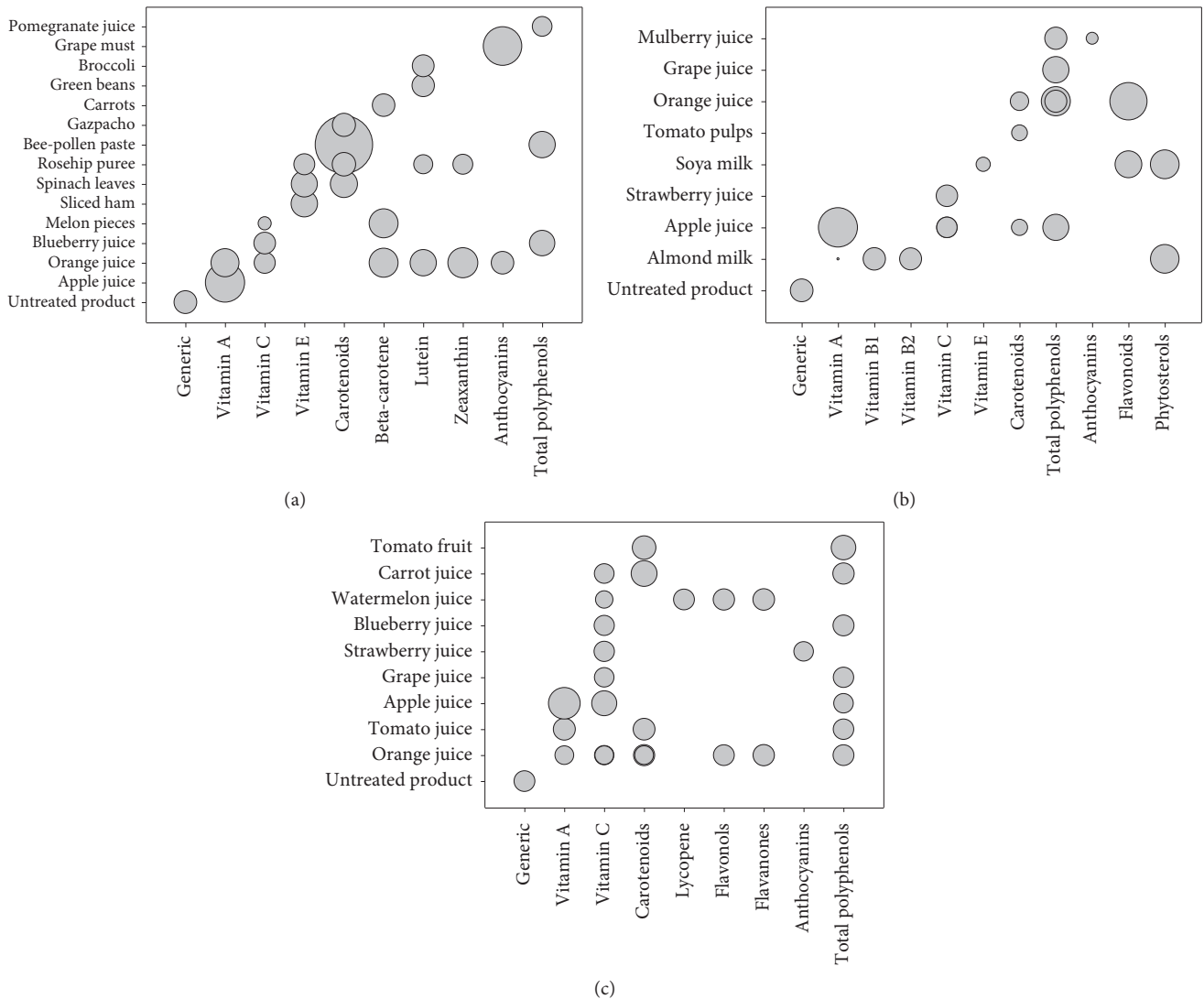


FIGURE 2: Effect of various treatments of different food products on the concentration of different bioactive compounds, with respect to a generic compound in an untreated product. (a) High-pressure processing; (b) high-pressure homogenization; (c) pulsed electric fields. Size of each bubble is related to the concentration of the bioactive compounds after the nonthermal treatment, with respect to the initial concentration.

In the dairy industry, homogenization has been extensively used for the stabilization of food emulsions and the disruption of fat globules. The higher operating pressures of HPH/UHPH treatments enable also the direct microbial inactivation, the disruption of smaller particles, and the modification of proteins or other food constituents [124]. Figure 2(b) depicts the effects of HPH/UHPH treatment of different liquid food products on the concentration of different bioactive compounds.

For example, both HPH and UHPH treatments reported to induce a significant reduction of the suspended particle size distribution in juices [121, 125, 126] and the juice viscosity [125, 127, 128]. Additionally, the color attributes of HPH-treated juices are not significantly altered, in comparison with the untreated product [121, 122, 125], whereas the cloudiness and opalescence stability are significantly improved [123, 126].

However, during pressure homogenization treatments, due to the significant temperature rise occurring in the homogenization valve, most of thermosensitive compounds, such as vitamins, carotenoids, and anthocyanins, are degraded to a higher extent than that by HPP. For example, when almond milk was treated at 200 or 300 MPa and very high inlet temperature (55–75°C), in order to obtain a microbiologically stable product, vitamin A was almost completely degraded [129]. However, in cloudy apple juice, a treatment, carried out at 100–175 MPa for 3–5 passes and an inlet temperature of 10–35°C, caused a significant increase in the content of vitamin A in the juice, with respect to the untreated product, due to the disruption effect on suspended vegetable cells.

Vitamins B1 and B2 were slightly reduced in almond milk treated for a single UHPH pass at 350 MPa and an inlet temperature of 40°C [130]. UHPH treatment caused a slight

reduction in vitamin C in strawberry juice treated at higher pressure (205 MPa) for 3 passes at an inlet temperature of 20°C [131]. The vitamin E content in soya milk was significantly affected by an UHPH treatment carried out at 200 or 300 MPa and very high inlet temperature (55–75°C), required to obtain a microbiologically stable product [132].

The total carotenoid content also exhibited a significant decrease upon HPH/UHPH processing, independently on the food matrix. In apple juice, treated for a single pass at pressures between 100 and 300 MPa and inlet temperatures of 10–20°C, a reduction in carotenoids of 30% was observed [133]. In orange juice, treated at the same conditions, the carotenoids decreased about 20% [134]. In tomato pulp, processed, because of its higher viscosity, at lower HPH pressures (20–100 MPa) for 1 pass and an inlet temperature of 4°C, a decrease in the total carotenoids of about 30% was observed [135].

In contrast, the total polyphenols were well preserved by pressure homogenization treatments, independently on the food matrix. For example, in apple juice, treated for a single pass between 100 and 300 MPa and an inlet temperature of 10–20°C, the total polyphenols slightly increased [133]. In orange juice, treated under the same conditions, the total polyphenols remained constant [134]. In orange juice and grape juice, treated at 250 MPa and room temperature, the total polyphenols slightly increased, with respect to untreated juices [136]. Similarly, mulberry juice, treated by UHPH at 200 MPa for 3 passes and an inlet temperature of 4°C, the total polyphenols remained constant [137]. Conversely, the same treatment had a detrimental effect on the anthocyanins of mulberry juice, with an observed reduction of almost 50% [137].

Remarkably, the content of flavonoids exhibited a significant increase upon UHPH processing. For example, in soya milk, despite the treatment at high inlet temperatures (55–75°C, with a single pass at 200 or 300 MPa), the flavonoids increased of about 20% [132]. In orange juice, treated in the pressure range (100–300 MPa) but at significantly lower inlet temperatures (10–20°C), an even more significant increase in the content of flavonoids was observed, in comparison with untreated juice [134].

Pulsed electric field (PEF) technology is recognized to be a technique able to cause a significant microbial inactivation in beverages, while causing only a minimal impact both on the quality properties and on the content of health-beneficial compounds. This is mainly due to the low treatment temperature: although the intensity of PEF treatments might reach electric fields intensities up to 40 kV/cm and a total energy delivered to the product of 40–100 kJ/L [61], the product temperature can be maintained below 40°C [138, 139].

Figure 2(c) clearly shows that, in different fruit and vegetable juices, no significant decreases are observed among the main health-beneficial compounds, including vitamins, carotenoids, and phenolic compounds. In contrast, in some cases, a significant increase is observed, which can be attributed to the enhanced extraction of bioactives from the vegetable cells [140].

In particular, vitamin A is observed to only slightly decrease of less than 10% in orange juice [141], but to significantly increase in apple [61] and in tomato juice [138].

Similarly, the vitamin C retention was always very high, comprised between 90 and 100%, independently on the food matrix [139, 142–145]. In the case of carotenoids, the PEF treatments did not cause any significant decrease in concentration in orange juice [141, 146], tomato juice [138], and watermelon juice [144] and a measurable increase (+25%) in carrot juice [147].

In addition, no significant variation was observed for flavonols in orange [148] or watermelon juice [144], for flavanones in orange juice [146, 148] or watermelon juice [144], for anthocyanins in strawberry juice [139], for total polyphenols in apple [145], or in orange [148], in grape [145], in blueberry [142], in tomato [139], and in carrot juices [147].

Ultrasound is thought to enhance the destabilization of casein micelles in milk [149]. This can be used to advantage in the coagulation of various milk sources, for example, goats' milk and reconstituted milk. Goats' milk is known for its weaker coagulation abilities as compared to cows' milk. The use of ultrasound treatment prior to addition of rennet resulted in smaller and more uniform particle sizes in the coagulant formed [150, 151]. Ultrasonically treated milk and yogurt samples also showed an increase in the gel firmness, coagulum strength, final storage modulus, cohesiveness, and water holding capacity [152]. These factors were stated to be due to a decrease in the soluble proteins and an increase in insoluble high-molecular-weight coaggregates formed as a result of protein denaturation on sonication [153, 154].

Monteiro et al. [155] noticed that ultrasound energy affects the physical properties of chocolate milk and the subsequent size distribution of fat globules and resultant rheological behavior of the treated sample; however, the bioactive compounds present and the nutritional quality of the product were still maintained. Changes to food materials is dependent in the first case on the frequency employed for sonication whether physical or mechanical changes are required, thus employing lower frequencies, or whether chemical changes are required where higher frequencies would be of more benefit.

Supercritical drying with the use of supercritical fluids (CO₂) is used as an alternative process to conventional drying techniques [156]. Carbon dioxide (CO₂) at high pressures (7.0 to 30.0 MPa) or in supercritical phase (above 31°C, 7.3 MPa) is considered as a novel nonthermal technology [157]. This preservation technology achieves inactivation of microorganisms and also meets consumers' demands for a product with high nutritional and sensory qualities [158]. Its main advantage is operation at relatively low temperature that avoids the thermal effects of traditional heat preservation, retaining the food freshness [159].

In food application field, nonthermal plasma (NTP) comes in various ways, from food surface application to direct in liquid food application. NTP is effective and causes minor harm to the exposed materials, such as biological samples or processed foods or packaging materials [160]. Researches have been mostly exploring plasma effect on inactivation of microorganisms [161, 162], but lately, plasma effect on food ingredients becomes focus topic due to combined physicochemical effects and complexed food

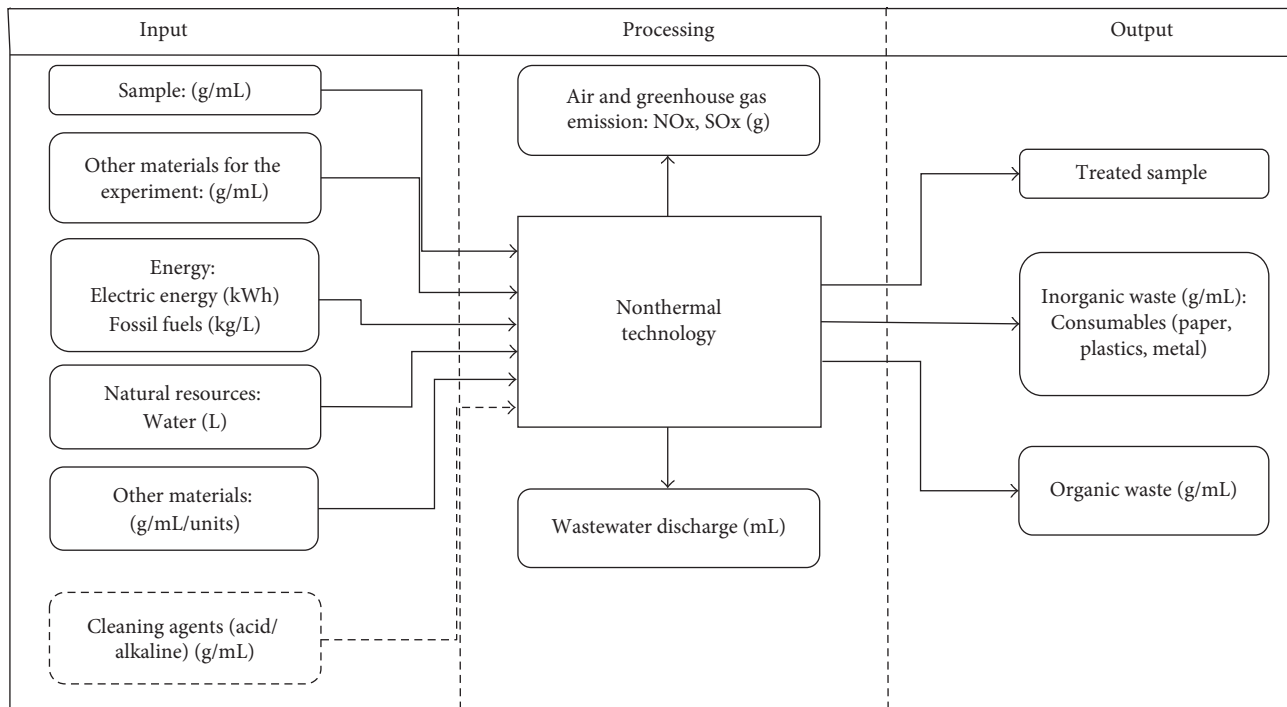


FIGURE 3: Generic model of using nonthermal technologies. Rounded rectangles outlined in solid lines present direct inputs and outputs; rounded rectangles outlined in dashed lines present inputs/outputs not directly related to nonthermal technologies.

structure. Plasma application in/on food brings another perspective of possible negative effects on phenolic compounds due to production of oxidative species.

Grzegorzewski et al. [163] have noted a degradation of phenolic compounds in lamb's lettuce after NTP treatment, but Misra et al. [164] have reported that cold plasma treatments had no significant effect on anthocyanins in strawberries and that, at the same time, phenolic acids have remained unchanged. Regarding liquid food, like fruit juices, an increase in total phenolic content [165] and anthocyanin content [166] in pomegranate juice after plasma treatment has been observed, as well as an increase in anthocyanin and phenolic acid contents in sour cherry Marasca juice [167] has been observed. Lukić et al. [168] report effects of plasma treatment on wines (red wine Cabernet Sauvignon and white wine Graševina) which have resulted in slight changes of chromatic characteristics and in reduction of phenolic compounds in both red and white wines, including total phenolics, total anthocyanins, total tannins, and certain free anthocyanins, while the concentrations of the most individual phenolic acids and flavan-3-ols slightly increased. These results led to new opening field of plasma treatment as a new extraction method due to improvement in the extraction of phenolic and other compounds.

5. Importance of Analyzing Environmental Impacts of Nonthermal Food Technologies

Besides obtaining safe products with high quality with nonthermal technologies, food processors are increasing their interest in reducing the environmental footprint of the

products and the processing cost [169]. However, analysis and comparison of environmental impacts of nonthermal technologies pose a challenge mainly because of the differences in the scale of the facilities and food processed (meat, egg, fruit, vegetables, liquid food, etc.). In most of the cases, these techniques are not implemented in large-scale industrial facilities and are often studied on lab scale or pilot level without deep analysis of the complete process [170].

Technologies, such as pulsed electric field treatment or high-pressure treatment, not only achieve microbial inactivation under mild conditions or inactivate certain enzymes and prevent undesired changes in food but also decrease processing time and decrease energy consuming [6, 171].

In analyzing nonthermal technologies, it is common to use the life cycle assessment (LCA) approach. It is a scientific method that includes mapping the process, setting the scope and boundaries, collecting data, calculating, evaluating, and interpreting the results with the aim to propose environmental improvements [172]. Hospido et al. [170] stress the difficulties in evaluating environmental impact of these technologies in terms of (i) the lack of real data for the inventory phase, which is often based on lab-scale information or theoretical data; (ii) the definition of the functional unit for comparative studies since new products or processes might have unique properties; and (iii) that manufacture of products or processes can be expected to start several years ahead and assumptions on surrounding systems will be required. Once the technologies are transferred from labs to real production plants, novel processing technologies can be compared with existing commercial alternatives and environmental hotspots can be identified [173].

Evaluation of environmental impact of novel technologies is usually performed using a partial life cycle assessment (LCA) approach. It included mapping the process of novel food treatments, setting scope and boundaries as lab scale, collecting and calculating data, and evaluating the results [172]. Functional unit (FU) as an output reference may be set as 1 kg/1 L of treated food product. Figure 3 depicts the generic system boundaries.

There are some comparative LCA studies of conventional and novel technologies. Pardo and Zufia [174] evaluated the environmental impacts of some traditional and novel food preservation technologies with the aim to contribute to the development of more sustainable food products. Some general improvements were identified, and environmental criteria were provided in order to select the more adequate preservation method when designing new food products. Valsasina et al. [175] compared ultrahigh-pressure homogenization with common thermal treatment for milk. The upscaling showed a decrease in carbon footprint up to 88% achievable with improvements in efficiency.

Aganovic et al. [176] studied the energy balance and LCA of pulsed electric fields and high-pressure processing technologies in comparison with conventional thermal processing applied to the preservation of tomato and watermelon juices. However, at a pilot scale, both pulsed electric field and high-pressure processing technologies presented higher energy consumption expressed per liter of juice, indicating the necessity for further optimization of the process.

6. Future Challenges of Novel Nonthermal Technologies and Conclusion

Legislation on hygienic design of food-processing equipment is rather vague [34]. Considering that there are a large number of different types of standards and regulations related to hygienic design and due to the redundancy of many requirements, a compact tool for evaluating novel technologies is more than needed [33].

In the future, studies related to comparison of environmental impacts of novel and conventional techniques will need to go in two directions: (i) improving the environmental performance of nonthermal technologies *per se*, and (ii) comparing environmental aspects of nonthermal and conventional technologies, along with weighting other factors such as quality of the final product or investment costs [2].

Conflicts of Interest

The authors confirm that there are no conflicts of interest associated with this publication.

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Research Article

Effect of Differing Ingredients and Packaging Technologies on the Color of High-Pressure Processed Ground Beef

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High-pressure processing (HPP) is a nonthermal pasteurization technique to control pathogens, like *Escherichia coli*. However, color changes in raw beef induced by HPP restrict its use within the beef industry. The objectives of this study were to investigate the effects of adding curing agents (nitrite) and packaging with or without reducing compounds (ascorbic acid/erythorbate) on color retention in high-pressure processed ground beef. Color was measured (CIE $L^*a^*b^*$) before HPP and on days 3, 7, 12, 14, 19, and 21 after HPP. Statistical analysis (SAS GLIMMIX) was run to identify the main effects of adding curing agents, packaging, and reducing agents on color retention. HPP resulted in a detrimental effect on the color of the beef patties for all treatments. Lightness and yellowness increased ($P < 0.001$) and redness decreased ($P < 0.001$) after high-pressure processing. The effect remained the same throughout the course of the study. However, there were less color changes in samples treated with reducing compounds. Both synthetic and natural sources of nitrite and ascorbic acid/erythorbate performed similarly in terms of their ability to maintain redness. Treatments leading to formation of nitrosylmetmyoglobin (Fe^{3+}) had less severe color change compared to the treatments leading to the generation of nitrosylmyoglobin (Fe^{2+}).

1. Introduction

A major challenge faced by the ground beef processors is microbial contamination such as *E. coli* O157:H7 and other Shiga toxin producing *E. coli* (STEC). Sanitary handling, preharvest washing, and spraying the carcass with organic acids reduce the risk but do not completely eliminate contamination [1]. In ground beef and other nonintact beef products, STECs are considered an adulterant by the USDA [2]. These products are at a greater food safety risk as pathogens can be introduced throughout the product, rather than just on the surface. High-pressure processing (HPP) is a nonthermal pasteurization technique where pressure treatment between 300 and 800 MPa kills bacteria [3] by cell wall/spore coat rupture [4] or denaturation of critical proteins/enzymes [5, 6]. The process is most effective on Gram negative bacteria followed by yeasts/molds, Gram positive bacteria, and spores [4]. *Salmonella*, *Listeria*, and

E. coli are the major meat pathogens which can be effectively controlled by HPP. A 2.0 to 6.0 log CFU/g reduction of these pathogens is achievable by HPP treatment [7–9].

Several HPP-treated food products are in the market including fruit jellies and jams, fruit juices, pourable salad dressings, raw squid, rice cakes, foie gras, ham, and guacamole [3]. However, the use of HPP on raw meat products is uncommon due to high-pressure-induced protein denaturation and discoloration [10–15]. Therefore, it is important to find ways to stabilize the bright red color (oxymyoglobin) of fresh meat to develop a HPP-based pasteurization techniques for raw ground beef products. Effectiveness of HPP to control pathogens in cooked and uncooked meat and poultry items has been studied in great detail [7–9, 12, 14]. However, there are only very few reports available about the effect of HPP on the appearance of raw beef [3, 13, 16, 17].

Use of curing agents, such as nitrite salts, is well known to retain the bright red meat color. Nitrite salt generates

TABLE 1: Least square means (\pm SE) for main effect of high-pressure processing on color (L^* , a^* , b^*) and change in color (ΔE) during storage of ground beef patties.

Color traits	HPP (MPa/min)	Color values ¹					
		Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L^*	0/0	40.78 \pm 0.48 ^b	42.20 \pm 0.34 ^c	43.13 \pm 0.30 ^c	43.73 \pm 0.38 ^c	42.06 \pm 0.43 ^b	43.31 \pm .034 ^c
	450/3	53.58 \pm 0.50 ^a	54.49 \pm 0.36 ^b	54.87 \pm 0.31 ^b	55.09 \pm 0.39 ^b	55.30 \pm 0.45 ^a	55.26 \pm 0.36 ^b
	600/3	54.53 \pm 0.48 ^a	55.62 \pm 0.34 ^a	56.66 \pm 0.30 ^a	56.2 \pm 0.38 ^a	56.45 \pm 0.43 ^a	55.61 \pm 0.34 ^{ab}
	600/6	53.47 \pm 0.48 ^a	55.84 \pm 0.34 ^a	55.98 \pm 0.30 ^a	56.20 \pm 0.38 ^a	56.36 \pm 0.43 ^a	56.29 \pm 0.34 ^a
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
a^*	0/0	22.50 \pm 0.55 ^a	21.98 \pm 0.56 ^a	19.46 \pm 0.46 ^a	19.33 \pm 0.44 [†]	21.04 \pm 0.50 [†]	19.39 \pm 0.62 ^a
	450/3	21.33 \pm 0.57 ^{ab}	18.18 \pm 0.58 ^b	16.56 \pm 0.48 ^b	14.91 \pm 0.46 [†]	16.02 \pm 0.52 [†]	16.38 \pm 0.64 ^b
	600/3	18.17 \pm 0.55 ^b	16.35 \pm 0.56 ^c	14.31 \pm 0.46 ^c	14.67 \pm 0.44 [†]	14.62 \pm 0.50 [†]	14.68 \pm 0.62 ^{bc}
	600/6	20.43 \pm 0.55 ^c	15.81 \pm 0.56 ^c	14.46 \pm 0.46 ^c	14.76 \pm 0.44 [†]	13.77 \pm 0.50 [†]	13.21 \pm 0.62 ^c
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b^*	0/0	8.95 \pm 0.17 ^d	9.50 \pm 0.18 ^c	8.70 \pm 0.14 ^c	8.34 \pm 0.16 ^c	9.20 \pm 0.20 ^b	8.90 \pm 0.17 [†]
	450/3	11.14 \pm 0.18 ^c	10.67 \pm 0.19 ^b	10.67 \pm 0.15 ^b	10.44 \pm 0.16 ^b	11.39 \pm 0.20 ^a	11.00 \pm 0.18 [†]
	600/3	11.67 \pm 0.17 ^b	11.32 \pm 0.18 ^a	11.27 \pm 0.14 ^a	11.39 \pm 0.16 ^a	11.95 \pm 0.20 ^a	11.55 \pm 0.17 [†]
	600/6	12.34 \pm 0.17 ^a	11.12 \pm 0.18 ^{ab}	11.44 \pm 0.14 ^a	11.49 \pm 0.16 ^a	11.72 \pm 0.20 ^a	11.65 \pm 0.17 [†]
	<i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ΔE	450/3	13.14 \pm 0.89	13.29 \pm 0.54	12.84 \pm 0.62	12.95 \pm 0.52	15.07 \pm 0.73	13.17 \pm 0.62
	600/3	15.00 \pm 0.85	14.90 \pm 0.52	14.92 \pm 0.59	14.01 \pm 0.50	16.54 \pm 0.71	13.98 \pm 0.60
	600/6	13.90 \pm 0.85	15.32 \pm 0.52	14.34 \pm 0.59	14.14 \pm 0.50	16.57 \pm 0.71	14.94 \pm 0.60
	<i>P</i> value	0.322	0.026	0.056	0.209	0.259	0.134

^{a-c}LS means in a column and within a color trait with a common superscript are similar ($P > 0.05$). [†]A significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day. ¹Each experiment was carried out in triplicate.

HNO₂ and is reduced to NO in the presence of reducing agents or by other endogenous pathways [18, 19]. NO reacts with myoglobin in anaerobic and aerobic conditions to generate bright red nitrosylmyoglobin and nitrosylmetmyoglobin, respectively. These two are more stable than oxymyoglobin and thus impart greater color stability. Reducing agents, such as erythorbate or ascorbic acid, increase the nitrosylation rate and have shown to improve color stability in raw ground beef [20]. Excess reducing agent plays a dual role by inhibiting lipid oxidation and by increasing stability of cured meat color through shifting the equilibration between nitrosylmyoglobin and oxymyoglobin [21].

With the recent trend of using natural food ingredients, synthetic nitrite salt has been greatly replaced by plant based nitrite sources, such as celery juice powder (CJP), swiss chard, spinach, and broccoli [22]. CJP is advantageous due to absence of any strong color or flavor [23]. Similarly, cherry powder (CP) is a rich source of ascorbic acid and potential alternative to sodium ascorbate/erythorbate [24, 25]. The objective of this study was to study the effects of adding different nitrosylating agents in the presence and absence of reducing agents and aerobic and anaerobic packaging on the color stability of HPP-treated ground beef patties.

2. Materials and Methods

2.1. Patty Preparation. Boneless, denuded USDA Select beef top rounds were ground through 1.27 cm and 0.32 cm grinding plates (Model 4732, Hobart Manufacturing, Troy, OH) and subdivided into six batches of 2.27 kg. The fine ground beef was mixed using a commercial kneader-mixer (RM-20, Manica USA, St. Louis, MO) with the following ingredients to convert myoglobin to different nitrosylmyoglobin

states with or without the addition of reducing compounds (sodium erythorbate or ascorbic acid from cherry powder). The treatments (T1–T6) are as follows:

T1: sodium nitrite 156 ppm/vacuum packaging (VP; anaerobic packaging)

T2: sodium nitrite 156 ppm + sodium erythorbate 547 ppm/VP

T3: celery juice powder (VegStable 506, Florida Food Products, Inc., Eustis, FL; to add 100 ppm sodium nitrite equivalent)/VP

T4: celery juice powder (equivalent to 100 ppm nitrite) + 0.43% cherry powder (VegStable 515, Florida Food Products, to add 469 ppm ascorbic acid)/VP

T5: sodium nitrite 156 ppm/oxygen permeable wrap (OPW; aerobic packaging)

T6: sodium nitrite 156 ppm + sodium erythorbate 547 ppm/OPW.

Four 113 g patties were formed from each portion. Patties were formed using a 10.92 cm diameter hand operated patty press. All T1, T2, T3, and T4 patties were vacuum packed (Clarity 3 mil standard barrier nylon/polyethylene pouches, Bunzl Processors Division, North Kansas City, MO; OTR = 0.007 ml/cm²/24 hr at 23°C and 0% relative humidity) using the vacuum sealer (Multivac Model C500; Multivac Inc., Kansas City, MO). Treatments T5- and T6-treated patties were placed on foam trays (13.3 × 25.6 × 1.4 cm, Styro-Tech, Denver, CO) and overwrapped with oxygen permeable polyvinyl chloride (Prime Source PSM 18 #75003815, Bunzl Processors Division, North Kansas City, MO; oxygen transmission rate = 2.25 ml/cm²/24 hr at 23°C and 0% relative humidity; water vapor transfer rate = 496 g/m²/24 hr at 37.8°C and 90%

TABLE 2: Least square means (\pm SE) for main effect of myoglobin state (Mb) on color (L^* , a^* , b^*) and change in color (ΔE) during storage of ground beef patties.

Color traits	Mb state ¹	Color values ²					
		Day 3	Day 7	Day 12	Day 14	Day 19	Day 21
L^*	T1	50.64 \pm 0.62	52.25 \pm 0.44	53.55 \pm 0.39	53.06 \pm 0.49	53.32 \pm 0.56	53.40 \pm 0.44 ^a
	T2	51.17 \pm 0.58	52.72 \pm 0.42	52.63 \pm 0.37	52.83 \pm 0.46	51.59 \pm 0.53	52.65 \pm 0.42 ^a
	T3	49.74 \pm 0.58	51.61 \pm 0.42	52.58 \pm 0.37	53.49 \pm 0.46	52.49 \pm 0.53	52.84 \pm 0.42 ^a
	T4	50.37 \pm 0.58	51.86 \pm 0.42	52.09 \pm 0.37	52.23 \pm 0.46	52.01 \pm 0.53	52.28 \pm 0.42 ^{ab}
	T5	51.86 \pm 0.58	52.08 \pm 0.42	53.04 \pm 0.37	52.99 \pm 0.46	53.63 \pm 0.53	53.17 \pm 0.42 ^a
	T6	49.75 \pm 0.58	51.70 \pm 0.42	52.07 \pm 0.37	52.25 \pm 0.46	52.20 \pm 0.53	51.37 \pm 0.42 ^b
	P value	0.095	0.455	0.065	0.357	0.073	0.023
a^*	T1	20.51 \pm 0.72 ^b	17.28 \pm 0.72 ^b	13.42 \pm 0.60 ^c	14.49 \pm 0.57 [†]	13.99 \pm 0.65 [†]	13.88 \pm 0.80 ^b
	T2	23.29 \pm 0.68 ^a	19.94 \pm 0.68 ^a	18.34 \pm 0.57 ^{ab}	18.67 \pm 0.54 [†]	19.42 \pm 0.61 [†]	17.80 \pm 0.76 ^a
	T3	21.38 \pm 0.68 ^{ab}	17.28 \pm 0.68 ^b	14.23 \pm 0.57 ^c	13.21 \pm 0.54 [†]	14.13 \pm 0.61 [†]	14.47 \pm 0.76 ^b
	T4	23.13 \pm 0.68 ^a	20.53 \pm 0.68 ^a	19.83 \pm 0.57 ^a	19.23 \pm 0.54 [†]	18.90 \pm 0.61 [†]	18.14 \pm 0.76 ^a
	T5	14.31 \pm 0.68 ^c	14.45 \pm 0.68 ^c	13.71 \pm 0.57 ^c	12.50 \pm 0.54 [†]	13.20 \pm 0.61 [†]	13.28 \pm 0.76 ^b
	T6	21.03 \pm 0.68 ^b	19.00 \pm 0.68 ^{ab}	17.65 \pm 0.57 ^b	17.42 \pm 0.54 [†]	18.52 \pm 0.61 [†]	17.93 \pm 0.76 ^a
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
b^*	T1	11.00 \pm 0.23 ^a	10.47 \pm 0.24	10.41 \pm 0.18	10.60 \pm 0.20	11.52 \pm 0.25	11.27 \pm 0.22 [†]
	T2	11.34 \pm 0.21 ^a	10.88 \pm 0.23	10.58 \pm 0.17	10.33 \pm 0.19	10.89 \pm 0.24	10.10 \pm 0.21 [†]
	T3	11.45 \pm 0.21 ^a	10.72 \pm 0.23	10.42 \pm 0.17	10.26 \pm 0.19	11.51 \pm 0.24	10.89 \pm 0.21 [†]
	T4	11.48 \pm 0.21 ^a	10.97 \pm 0.23	10.84 \pm 0.17	10.59 \pm 0.19	10.70 \pm 0.24	10.39 \pm 0.21 [†]
	T5	9.91 \pm 0.21 ^b	10.46 \pm 0.23	10.39 \pm 0.17	10.41 \pm 0.19	11.06 \pm 0.24	11.42 \pm 0.21 [†]
	T6	10.96 \pm 0.21 ^a	10.41 \pm 0.23	10.47 \pm 0.17	10.31 \pm 0.19	10.71 \pm 0.24	10.60 \pm 0.21 [†]
	P value	<0.001	0.362	0.431	0.724	0.056	<0.001
ΔE	T1	14.10 \pm 1.30	14.03 \pm 0.79 ^{ab}	13.3 \pm 0.90 ^{bc}	14.54 \pm 0.76 ^b	18.75 \pm 1.08 ^a	16.85 \pm 0.91 ^a
	T2	12.49 \pm 1.20	12.24 \pm 0.73 ^b	12.46 \pm 0.84 ^c	12.28 \pm 0.70 ^c	16.07 \pm 1.00 ^{ab}	13.13 \pm 0.84 ^{bc}
	T3	12.65 \pm 1.20	15.97 \pm 0.73 ^a	15.20 \pm 0.84 ^{ab}	17.60 \pm 0.70 ^a	16.63 \pm 1.00 ^{ab}	13.80 \pm 0.84 ^b
	T4	13.88 \pm 1.20	14.17 \pm 0.73 ^{ab}	13.08 \pm 0.84 ^{bc}	11.78 \pm 0.70 ^c	15.29 \pm 1.00 ^{bc}	14.25 \pm 0.84 ^b
	T5	13.36 \pm 1.20	15.26 \pm 0.73 ^a	16.51 \pm 0.84 ^a	13.34 \pm 0.70 ^{bc}	16.56 \pm 1.00 ^{ab}	14.92 \pm 0.84 ^{ab}
	T6	17.61 \pm 1.20	15.34 \pm 0.73 ^a	13.67 \pm 0.84 ^{bc}	12.65 \pm 0.70 ^{bc}	13.05 \pm 1.00 ^c	11.24 \pm 0.84 ^c
	P value	0.055	0.015	0.015	<0.001	0.015	0.003

^{a-c}LS means in a column and within a color trait with a common superscript are similar ($P > 0.05$). [†]A significant myoglobin state by HPP treatment interaction ($P < 0.05$) for the color trait within the day. ¹T1: sodium nitrite 156 ppm/vacuum packaging (VP; anaerobic packaging); T2: sodium nitrite 156 ppm + sodium erythorbate 547 ppm/VP; T3: celery juice powder to add 100 ppm sodium nitrite equivalent/VP; T4: celery juice powder (equivalent to 100 ppm nitrite) + 0.43% cherry powder to add 469 ppm ascorbic acid/VP; T5: sodium nitrite 156 ppm/oxygen permeable wrap (OPW; aerobic packaging); T6: sodium nitrite 156 ppm + sodium erythorbate 547 ppm/OPW. ²Each experiment was carried out in triplicate.

relative humidity). All patties were stored at 4°C for two days to allow for conversion to nitrosylmyoglobin (T1–T4) and nitrosylmetmyoglobin (T5–T6). After 48 hours, T5 and T6 were vacuum packaged just prior to HPP treatment. Three independent replications were produced.

2.2. High-Pressure Processing Treatment. Samples were processed using a 55 L HPP unit (Hiperbaric 55, Miami, FL) located in the food grade lab of the Food Processing Center, University of Nebraska Lincoln [26]. Processing of HPP-treated samples was performed at three different combinations of pressure, and the hold time (600 MPa/3 minutes, 600 MPa/6 minutes, and 450 MPa/3 minutes) that were chosen based on their effectiveness to reduce pathogens, according to previous research. During the course of the study, all samples were stored at 4°C to better simulate commercial refrigerated storage.

2.3. Colorimetry. Color of the patties was measured (CIE $L^*a^*b^*$) through the vacuum pouch prior to HPP and on days 3, 7, 12, 14, 19, and 21 after HPP [26]. A colorimeter

(CR-300, MINOLTA, Japan) was used to determine the instrumental color which uses diffuse D65 illumination, 8 mm viewing port, and 0° viewing angle (specular component included). The system was calibrated to the included white calibration plate covered in the vacuum pouch before each measurement period. The average of at least three measurements was taken from randomly selected areas on the patty surface. Change in color, ΔE , was calculated with respect to the control samples (non-HPP treated) within each of the six treatments:

$$\Delta E = \left[(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2 \right]^{1/2}, \quad (1)$$

where subscripts *i* and *f* represent before and after HPP treatment, respectively.

2.4. Statistical Analyses. Statistical analyses were run on color data (L^* , a^* , b^* , ΔE) using a statistical software package (SAS 9.4, SAS Cary, NC) to see the main effects of ingredient/packaging conditions (T1–T6) and HPP treatment and their interactions within each day of storage [26]. Treatment interaction and main effects were determined using the mixed mode general linear

model (PROC GLIMMIX). When significant ($P < 0.05$) interactions or main effects were identified, separation of least square means was conducted.

3. Results and Discussion

Regardless of the ingredients/packaging treatment, HPP had a detrimental effect on the color of the beef patties for all three pressure and time combinations (Table 1). Lightness (L^*) and yellowness (b^*) increased and redness (a^*) decreased ($P < 0.001$) due to HPP treatment for all days of storage. Within each day, color change with respect to control samples (ΔE) was similar ($P > 0.05$) for all three HPP conditions. Table 2 represents the effect of different ingredients/packaging on the color parameters. Within a particular day, all six differently treated samples had similar lightness (L^* , $P > 0.05$, except for day 21) and yellowness (b^* , $P > 0.05$, except for day 3 and day 21) but showed differences in redness (a^* , $P < 0.001$). Samples treated with reducing compounds (T2, T4, and T6) showed greater redness (higher a^*) than the counterparts without reducing compounds (T1, T3, and T5), and this pattern was maintained throughout the course of the study. Reduction of oxidized myoglobin (nitrosylmetmyoglobin) to nitrosylmyoglobin may be responsible for increasing the redness. Among the color parameters evaluated, a^* had an interaction of treatment (T1–T6) \times HPP effects ($P \leq 0.004$) for days 14 and 19 only (data not shown) and b^* had an interaction of treatment (T1–T6) \times HPP effects ($P = 0.012$) for days 21. On these days, treatments with reducing compounds had redness values that were more similar to the non-HPP treated control samples than treatments without reducing compounds which matches the significant main effect identified for a^* for all other days. On day 21, HPP-treated samples were more yellow than non-HPP treated samples. Others have reported that the addition of antioxidants containing cherry powder, a natural source of ascorbic acid, to ground beef resulted in greater red color in patties in simulated retail display [20]. Similar a^* values of T2 and T4 within a particular day signifies that both inorganic and plant-based sources of nitrite and reducing compounds had a similar influence on color. T1 had significantly higher a^* than T5 on day 3, but the difference became less profound during storage. Although immediately after HPP, nitrosylmyoglobin is more red, and it became less red and approached that of T5, likely due to the fact that nitrosylmetmyoglobin in T5 had already oxidized and it started with less red color. ΔE of T6 was significantly higher than ΔE of T2 immediately after HPP but gradually decreased during storage. This suggests that T6 changes color after HPP, but color changes lessened during shelf storage. This is most likely due to the reduction of nitrosylmetmyoglobin (brown) to nitrosylmyoglobin (red) by sodium erythorbate. In the absence of reducing agents (T1 versus T5), ΔE was similar throughout the course of the study.

4. Conclusions

While the addition of nitrite compounds alone did not stabilize ground beef color during HPP treatment, reducing compounds decreased the color change associated with HPP

treatment of ground beef. These findings may allow processors to progress toward the development of technologies that allow for the HPP treatment of raw ground beef without the negative color changes typically associated with the application of HPP.

Data Availability

All data related to this article are described in Tables 1 and 2. Persons interested in the raw data may contact the corresponding author to receive a copy.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Research Article

Effect of Temperature and Gamma Radiation on *Salmonella* Hadar Biofilm Production on Different Food Contact Surfaces

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Salmonella is a pathogen transmitted by foods and it is one of the most important target bacteria in food irradiation studies. Few works were carried out on the effectiveness of gamma radiation against biofilms formed by this bacterium. *Salmonella* can form a biofilm on different material surfaces. The physicochemical properties of surfaces and environmental factors influence the adhesion of this pathogen. The present study investigated the effect of gamma radiation (1 and 2 kGy) and temperature (28°C and 37°C) on the development of *Salmonella* Hadar biofilm on polyvinyl chloride (PVC), glass, cellophane paper (CELLO), and polystyrene (POLY). The obtained results indicated that biofilm production is surface and temperature dependent. In addition, biofilm formation decreased significantly after gamma irradiation at either 1 or 2 kGy doses. However, the *agfD* and *adrA* genes expression did not demonstrate significant decrease. This work highlighted that gamma radiation treatment could reduce the biofilm formation of *Salmonella enterica* serovar Hadar on different food contact surfaces.

1. Introduction

Biofilm is known as sessile microbial communities attached to substances and interfaces and to each other [1]. Bacterial cells, in biofilm, are embedded into a matrix which forms a physicochemical barrier against different environmental conditions. The biofilm generation depends on substrate type (hydrophobicity), bacterial cells (flagellar formation and motility), and environmental conditions such as temperature that plays a crucial role in the phenotypic change from planktonic form to sessile one [2]. Hu et al. [3] indicated that Curli fibers (aggregative fimbriae) and *agf* genes (known as *csg*) are involved in biofilm formation. Also, *bcsA*, *bcsB*, *bcsZ*, and *bcsC* genes' coding for cellulose synthesis plays a crucial role in biofilm formation. Moreover, fimbriae production is coregulated by a LuxR-type regulator gene, *agfD*, acting

indirectly on *adrA* gene for regulating cellulose production [4]. Several studies reported the ability of *Salmonella* spp. to develop biofilm on several materials (plastic, glass, stainless steel, and so on) and its resistance regarding antimicrobial agents under this physiological state [5, 6]. *Salmonella* spp. can produce biofilm on nutrient broth liquid-air interface [7, 8]. *Salmonella* spp. cultivated on Luria Bertani agar plates supplemented with Congo red express a phenotype known as "rdar" (red, dry, and rough) morphotype [7].

It was demonstrated that the *agfD* gene is necessary for the biofilm maturation and is responsible for all major matrix constituent's expression regulation [9]. Other studies indicated that this gene might be influenced by different stimuli [10].

Ionizing radiation (X-ray, gamma ray, and electron beam) is well-known as an effective method for destroying spoilage

and pathogenic microorganisms in foods [11]. The radiation of D_{10} values (dose of radiation necessary to reduce the population by 1 \log_{10} , or 90%) of various *Salmonella* strains was 0.65 kGy [12]. In addition, it was demonstrated that D_{10} value is depending on the isolates and substrate [13]. The first action of ionizing radiation is via oxygen and hydroxyl radicals, produced when the high-energy photons (X-ray and gamma) or electrons (electron beam) break water molecules [14]. These radicals harm cell membranes, protein structures, and nucleic acid strands. Irradiation also requires an orderly transfer of radiant energy into products and bacteria. We have previously described the effects of stress conditions (gamma radiation and static magnetic field) on *S. Hadar*. [13, 15]. In this work, we investigated the effect of ionizing radiation (gamma rays), temperature, materials, and the expression of *agfD* and *adrA* genes on *Salmonella* Hadar biofilm formation.

2. Material and Methods

2.1. Bacterial Strain and Growth Conditions. *Salmonella enterica* serovar Hadar was supplied by Pasteur Institute (Tunisia). It is a high antibiotic and radio resistant foodborne isolated from Turkish meat. *Salmonella* spp. were routinely cultivated on nutrient broth at 37°C for 24 hours.

2.2. Gamma Irradiation. Irradiation treatments were performed at the Tunisian semi-industrial Co_{60} gamma irradiation facility at a dose rate of 100 Gy/min. Doses were measured by the standard Fricke dosimeter. Irradiation was performed in 10 ml polyethylene tubes. Bacterial suspensions were irradiated at 1 and 2 kGy at room temperature (25°C). Control samples, nonirradiated, followed all the performed assays.

2.3. Biofilm Formation on Glass Tubes Assay. In order to investigate biofilm formation on glass tubes, pellicle formation was examined on an overnight culture incubated for 48 h at 28°C or 37°C, after gamma irradiation at 1 and 2 kGy doses according to the protocol proposed by Michal et al. [16].

2.4. Morphotypes on Congo Red Agar (CRA). The CRA method was done according to the protocol of Freeman et al. [17]. Media were prepared with Brain Heart Infusion (BHI) broth at 37 g/l, sucrose at 0.8 g/l, agar-agar at 10 g/l, and Congo red stain at 0.8 g/l. Plates of the medium were inoculated and incubated aerobically for 24 h at 37°C.

The biofilm positive strains produced black colored colonies and biofilm negative strains were pink colored [17].

2.5. The Microtiter Plate (MTP) Biofilm Assay. The MTP method was done according to the protocol of Møretrø et al. [18].

The biofilm production was carried out on different materials: polystyrene (POLY), polyvinyl chloride (PVC), glass (G), and cellophane paper (CELLO). For biofilm quantification, circular chips (1.3 cm diameter) of cellophane paper, polystyrene, and glass slides were used. These different materials were sterilized by autoclaving at 121°C for 15 min then placed into 96-well plates. Irradiated *Salmonella* (200 μ l of bacterial suspension) was diluted 1:100 in TSB. Then

200 μ l of this diluted suspension was transferred to each well. Plates were incubated for 24 h at 28°C and 37°C. After incubation, the broth was removed from each of the wells. Then, chips were transferred to a new 96-well microplate. Unfixed cells were removed by washing chips three times with phosphate buffer saline (PBS) and biofilm was dyed with crystal violet (1%). For colorimetric measurement, biofilm was resuspended in 200 μ l of glacial acetic acid during 15 minutes and plates were read on ELISA plate reader multiscan (Microplaque reader 680, Biorad) at a wavelength of 595 nm. BHI medium was used as a negative control [19].

2.6. Quantification of Biofilm-Producing Genes in *Salmonella*. *Salmonella* spp. were inoculated into (BHI) and incubated at 28°C or 37°C for 48 hours. Following incubation, 1 ml of the bacterial suspension was transferred into microtubes and centrifuged at 10,000g for 10 minutes. The pellet was used for RNA experiments.

2.7. RNA Extraction and Quantitative Reverse Transcriptase PCR (qRT-PCR). For total RNA extraction, Trizol Total RNA Isolation System (Sigma) was used according to the manufacturer's protocol. RNA was suspended in 30 μ l of sterile water after 15 minutes of incubation at 55°C. The final concentration measurement of RNA samples was performed using the NanoDrop ND1000 spectrophotometer (Thermo Scientific). RNA was extracted from both of irradiated and control samples. First strand cDNA synthesis was done using 1 μ g total RNA in 25 μ l reaction mixtures containing 10 mM deoxynucleoside triphosphate, 25 U of RNAsin, 5 μ l of a 5x buffer, 50 U of AMV-RT (Promega), and 0.5 μ g of random hexamers. The reaction mixture was incubated for 10 min at 25°C and then at 42°C for 30 minutes. Relative expression levels of *adrA* and *agfD* genes were fixed by RT-qPCR on cDNA achieved following the reverse transcription reaction. Amplification of both *adrA* and *agfD* genes was performed with primers as previously described by De Oliveira Débora et al. [20]. We used *recA* as reference gene for result normalization [21].

The qPCR amplifications were completed by mixing 10 ng cDNA with 12.5 μ l mixture of SYBR green Supermix (Bio-Rad) and 300 nM primers.

The fluorescence signal was revealed by a Mini Opticon real-time PCR instrument (Bio-Rad). Data shown are the means of three replicates.

2.8. Statistical Analysis. Average values of triplicates were provided, and the deviation was less than 5% of each value. Significance was estimated at a level of $P < 0.05$ using Student's test.

3. Results

3.1. *Salmonella*'s Colony Morphology and Biofilm Production. On CRA, untreated colonies appeared like black morphotype (Figure 1(a)). Concerning biofilm production assessed by microtiter plate (MTP), the results presented in Table 1 indicated that untreated *Salmonella* cultures preferentially adhere to PVC (O.D = 1.9) than to glass (O.D = 1.2). For

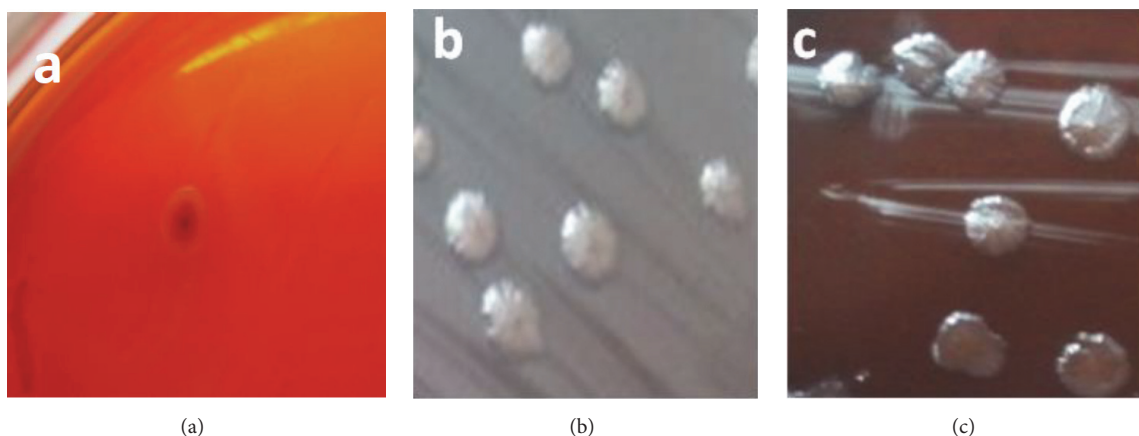


FIGURE 1: The morphotypes of *S. Hadar* before and after irradiation: (a) untreated black colonies; (b) bdar morphotype (1 kGy); and (c) rdar morphotype (2 kGy).

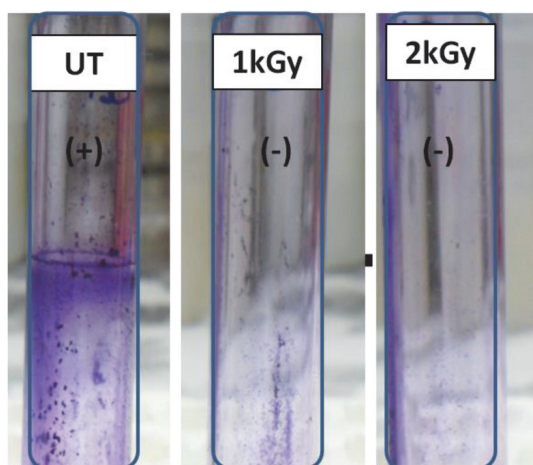


FIGURE 2: Biofilm production on glass tubes before (UT) and after gamma radiation at 1 kGy and 2 kGy doses. (-) indicates lack of pellicles and (+) indicates the presence of pellicles. UT: untreated bacteria.

the other materials (CELLO and POLY) adhesion is less important. Furthermore, for all the tested material biofilm production was enhanced at 28°C rather than at 37°C.

3.2. Effect of Gamma Radiation on Colony Morphology. For morphotype test, before treatment colonies were black (big biofilm producer) (Figure 1(a)). After gamma irradiation at 1 kGy, colonies appeared like bdar morphotype of blood agar (Figure 1(b)). However, at a dose of 2 kGy, the known morphotype, red, dry, and rough (rdar), was detected (Figure 1(c)), independently of the material pretested.

3.3. Effect of Gamma Radiation on Biofilm Formed Glass Surfaces. Biofilm production tested on the glass surface has demonstrated that, in the tube test, biofilm formation was considered as a ring of cells adhered to the glass wall at the air-liquid interface. Our results showed that untreated *S. Hadar* was able to produce biofilm on the air-liquid interface of glass

tubes. Interestingly, we noted the absence of this ring after gamma irradiation (Figure 2). Moreover, biofilm formation using microplate wells with glass slides was significantly ($P < 0.05$) affected by gamma rays; the optical density reflecting biofilm biomass decreased from 1.5 (control) to 0.8 (1 kGy) and 0.1 (2 kGy) (Table 1). Therefore, the ability to form biofilm was solely dependent on the applied gamma irradiation dose.

Results for cellophane paper are very different and biofilm production was significantly ($P < 0.05$) reduced to 37% (from O.D = 0.9 to O.D = 0.5) at 1 kGy and up to 94% (from O.D = 0.9 to O.D = 0.07) at 2 kGy doses (Table 2). The biofilm formation of polystyrene material was inhibited by 95% at 2 kGy and at 28°C (Table 2).

3.4. Effects of Gamma Radiation on *agfD* and *adrA* Gene Expression. The *agfD* and *adrA* genes are widely dispersed in *Salmonella* genus and are always linked with the aptitude to produce biofilms [20]. Both of analyzed genes were detected in *Salmonella Hadar*. The expressions of biofilm encoding genes *agfD* and *adrA* were calculated with respect to *recA* as a reference gene. The expression of the genes *agfD* and *adrA* showed significant upregulation after gamma radiation (1 and 2 kGy). This increase seems to be independent of the temperature. However, a significant dose effect was observed at 37°C (Figure 3).

4. Discussion

Gamma irradiation is an established technology of well-documented safety and efficacy for inactivation of pathogenic microorganisms such as *Salmonella* [22]. However, studies into the effectiveness of irradiation on biofilm-associated cells are lacking.

Many bacteria form aggregates at the bottom of containers and attach to the container surfaces in liquid media. However, some bacteria such as *Salmonella*, *Escherichia coli*, *Pseudomonas fluorescent*, and *Vibrio cholera* produce rigid or fragile pellicle structures at air-liquid interfaces [23]. Biofilm production by colonization of the air interface can facilitate and contribute to gas exchange while enabling the acquisition

TABLE 1: *Salmonella* Hadar biofilm inhibition in presence of incremental gamma radiation doses.

Materials	Dose (gamma irradiation)	Morphotype on CRA	Test tube (glass)	DO595 _(28°C)	DO595 _(37°C)
PVC	Ut	Black	Nf	1.9 ± 0.00*	2.5 ± 0.08
	1 kGy	Bdar	Nf	0.9 ± 0.04*	1.3 ± 0.05
	2 kGy	Rdar	Nf	0.08 ± 0.00*	0.1 ± 0.01*
Glass	UT	Black	++	1.2 ± 0.02*	1.5 ± 0.06
	1 kGy	Bdar	+	0.6 ± 0.00*	0.8 ± 0.03*
	2 kGy	Rdar	+	0.06 ± 0.00*	0.1 ± 0.02*
Cello	UT	Black	Nf	0.7 ± 0.01*	0.9 ± 0.04*
	1 kGy	Bdar	Nf	0.4 ± 0.00*	0.5 ± 0.03*
	2 kGy	Rdar	Nf	0.03 ± 0.00*	0.07 ± 0.00*
Poly	UT	Black	Nf	0.4 ± 0.00*	0.6 ± 0.05
	1 kGy	Bdar	Nf	0.2 ± 0.02*	0.2 ± 0.04*
	2 kGy	Rdar	Nf	0 ± 0.00*	0.04 ± 0.00*

UT: untreated; Nf: not found; * $P < 0.05$.

TABLE 2: Inhibition (%) of *Salmonella* Hadar biofilm formation, after gamma radiation exposure.

	PVC	Glass	CELLO	POLY
28°C				
1 kGy	54%	48%	37%	50%
2 kGy	96%	96%	95%	100%
37°C				
1 kGy	48%	50%	37%	57%
2 kGy	95%	94%	94%	93%

%: inhibition percentage.

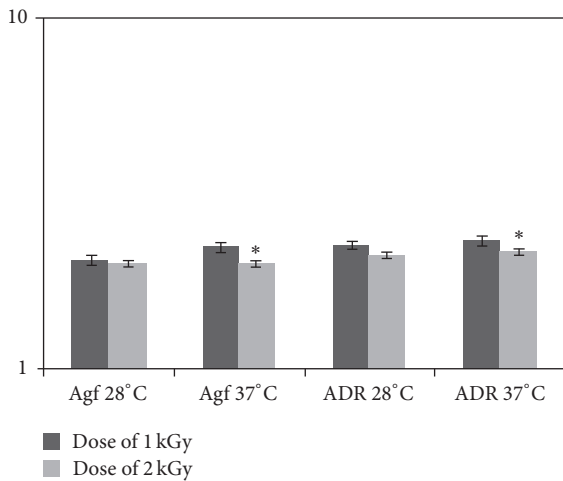


FIGURE 3: Effect of gamma radiation doses (1 and 2 kGy) on expression levels of agfD and adrA genes (* $P < 0.05$).

of nutrients and water from the liquid phase [24]. The biofilms at air-liquid interfaces can cause severe problems in industrial water systems [25].

Slime production was evaluated by culture on CRA plates. Untreated colonies were black colored. Black pigmented colonies on Congo Red Agar indicate biofilm producer strains. The modification of agar ingredients can also improve

phenotypic coloration [26]. Our results showed definite phenotypic changes following gamma radiation treatment at doses of 1 and 2 kGy. *S. Hadar* expressed bdar morphotype after 1 kGy treatment, then rdar morphotype was observed when treated by gamma radiation 2 kGy dose. Dubravka et al. [27] indicated that both rdar and bdar morphotypes are tolerant to disinfectants. Rdar morphology appears more tolerant to long-term *Salmonella* biofilm survival in a very dry environment, desiccation, and nutrient lack [28]. This result extends anterior results indicating that rdar morphotype indicates morphological adaptation to stress conditions and survival outside the host environment. The morphotype bdar has been suggested to be linked to *Salmonella* groups that do not need to survive for long periods in the host environment [29].

Biofilm production and adhesion on different surfaces were assessed and the same parameters described above were investigated such as (incubation temperature and gamma radiation doses). Bacterial adherence is influenced by the surface material, growth conditions, and the environmental factors including temperature [30]. These environmental factors play a vital role in the phenotypic change from planktonic cells to the sessile form [31].

Our results suggested that polystyrene is the material surface that presents the lower susceptibility to colonization. PVC avoided better the biofilm production at 37°C, followed by glass then cellophane. The high prevalence of biofilm production on PVC and glass at 37°C is agonizing because the permanency of *Salmonella* on these surfaces is in the starting of industrial processing and can be a valuable source of poultry contamination and a possible cause of foodborne diseases. Our results are in accordance with those reported by Hans et al. [4] who noticed that *Salmonella* adhered more easily to hydrophobic materials such as PVC than to stainless steel which is more hydrophilic. Mericarmen et al. [32] reported higher biofilm production by *Salmonella* on plastic than on stainless steel. Once a biofilm is formed, this could be a source of contamination for foods that is why protocols in food processing units should consider more *Salmonella's* biofilm removal. Thus, all our findings highlight

the hypothesis that biofilm forming abilities could be reduced with temperature decrease and increasing gamma radiation doses. To our knowledge, such a linkage has not been reported previously for *Salmonella*. However, Alonso et al. [33] stated that gamma radiation was effective in reducing the populations of biofilm-associated cells of *Salmonella enterica*. Treatment with gamma irradiation at the end of the production chain can be a good solution for biofilm removal. No correlation was observed between *agfD* and *adrA* expression and bacterial biofilm production. However, it was found that in *Salmonella* CsgD (acting via *agfD* and *adrA* genes) altered cell physiology to enable the generation of Curli, a process not yet identified in *Salmonella* [34]. Zakikhany et al. [35] identified a CsgD independent cellulose pathway, *adrA* independent. Ben Abdallah et al. [36] showed that there is no correlation between *sef* and *pef* *Salmonella* genes involved in adhesion and invasion and biofilm formation. More considerable attention must be given to the choice of potential contact surfaces and cleaning procedures when considering the efficient removal of biofilms. Indeed, biofilm formation is strongly affected by different environmental signals via a complex regulatory network. Comprehensive overview must be given to the comprehension of this genetic network and the interactions between its various components (*CsgD*, *RpoS*, *Crl*, *OmpR*, *IHF*, *CpxR*, *mlrA*, *BarA/SirA*, *Csr*, *PhoPQ*, *RstA*, *Rcs*, metabolic process, and quorum sensing) [4].

In summary, this work showed that *Salmonella enterica* serovar Hadar could adhere and form the biofilm on industrial surfaces such as polystyrene, PVC, glass, and cellophane paper. This could be a factor to be considered for the higher spoilage or/and disease transmission. Our study revealed that the biofilm production is dependent on temperature and the abiotic surface used. Moreover, gamma rays could be considered as an effective mean for *Salmonella* biofilms removal. Finally, it was proposed that *agfD* and *adrA* genes were not actively involved in *Salmonella* biofilm production.

Conflicts of Interest

The authors have declared no conflicts of interest.

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