PEF and UV combined system for pathogen microorganisms inactivation in liquid food products

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Abstract. Pulsed electrical field (PEF) treatment is a non-thermal food preservation technology based on the use of the electrical field in impulses applied in order to inactivate and control pathogen microorganisms in foods. This technology is highly appreciated for its ability to prolong the shelf life of the treated product without the use of heat and also for its ability to preserve the product’s sensory qualities and nutritional value as well as for the microbiological control of the treated products. This paper presents the PEF and UV treatment methods, or a combination between the two, for microbe inactivation in liquid products. The experiments were carried out using yeasts, lactic bacteria and acetic bacteria in the following systems: stand-alone treatments (PEF or UV) or in combination (UV+PEF or PEF+UV). The results of these experiments showed that one can obtain total inactivation of microorganisms using the combined UV+PEF system, thus leading to the possibility of increasing liquid food products quality as compared to the quality obtained using thermal pasteurization.

1. Introduction

Pulsed electrical field (PEF) treatment is a non-thermal technology for food preservation based on the use of a pulsed electrical field to inactivate pathogen microorganisms in food products and to control their content in products under treatment. This technology is highly appreciated for its ability to prolong shelf life without the use of heat, and also to preserve the sensory qualities and nutritional value of the product, as well as to microbiologically control the products under treatment. Literature in this field mentions that microorganism inactivation using PEF technology alone with naturally contaminated liquid food products as being difficult. Thus, the possibility of obtaining higher microbe inactivation rates was reported only in the case of combining PEF treatment with other effects, such as: heat, pressure or antimicrobial agents [1-2]. Paper [2] mentions the use of a combined PEF + UV treatment for the pasteurization of fresh apple juice. The results in the UV + PEF combined system were better from the microbial inactivation point of view as compared to the application of the PEF technology alone.

In the present work we present the PEF and UV treatment methods, or a combination between the two, for microbe inactivation in liquid products. The experiments were carried out using yeasts, lactic
bacteria and acetic bacteria in the following systems: stand-alone treatments (PEF or UV) or in combination (UV+PEF or PEF+UV). The aim is to develop a technology based on the combined treatment for the pasteurization of grape must and for the microbiological stabilization of wines.

2. Materials and methods

2.1. Materials under treatment
The experiments have been carried out on microorganism suspensions in distilled water sterilized by autoclaving for 30 minutes at 1 bar. The microorganisms to be tested were taken from the microorganism collection of Valea Calugareasca IC-DVV, from the category of microbes present in wines: for yeasts *Saccharomyces cerevisiae* was used, strain PN III-11, for lactic bacteria, *Oenococcus oenos* strain 149 and for acetic bacteria strain 11 of the *Acetobacter aceti* species. A series of consecutive replications on liquid mediums led to suspensions of microorganisms which, after several dilutions, were used to obtain certain inoculation titres.

Active microorganisms were highlighted using the microbiologic control method involving filtering through 0.45 μm pore membranes, followed by incubation on mediums specific to each type of microorganism: (a) for yeasts, agar based on yeast extract, peptone and glucose to which chloramphenicol was added; (b) For lactic bacteria, agar based on yeast extract, meat extract, peptone, sodium acetate, magnesium sulfate, glucose, to which acitidione was added; (c) For acetic bacteria, agar based on yeast extract to which acitidione and bromophenol blue were added. The experiments were carried out such that insight into following aspects is gained: (1) The influence of the duration of application of ultraviolet (UV) rays on microorganism inactivation; (2) The influence of the duration of application of pulsed electrical field (PEF) on microorganism inactivation; (3) The effect of the UV and PEF treatment, each alone and combined, on microorganism inactivation.

2.2. UV radiation processing
UV radiation exposure has been carried out in an UV rays water sterilizer with the following specifications: Working fluid – filtered water; Water flow = 0.1-0.3 l/min; Maximum power in the radiation chamber = 1 bar; UV wavelength = 254 nm; UV lamp power = 8 W; Power source = 220 V / 50Hz; Dimensions = 260 x 160 x 82 mm. Materials exposure to UV rays was carried out in lot conditions of 750 ml, from the products under treatment that have already been mentioned in section 2.1. The above mentioned apparatus allows continuous flow functioning. The materials under UV rays treatment were introduced in the PEF treatment chamber of 750 ml capacity. The HV impulses had the following characteristics: maximum impulse voltage: 30 kV; pulse front duration: 100 ns; pulse duration: 1 μs. The variable treatment duration is mentioned in the results section. The PEF system was cleaned and sterilized before and after each attempt.

2.3. High voltage impulses (PEF) processing
High voltage processing has been carried out in a concentric cylinder PEF treatment chamber with enhanced field area. The HV electrode was made from stainless steel with the diameter 60mm and the length 450mm. The materials under UV rays treatment were introduced in the PEF treatment chamber of 750 ml capacity. The HV impulses had the following characteristics: maximum impulse voltage: 30 kV; pulse front duration: 100 ns; pulse duration: 1 μs. The variable treatment duration is mentioned in the results section. The PEF system was cleaned and sterilized before and after each attempt.

3. Results
The tables and graphs below show the experimental results. Simple UV treatment led to a 36% - 62% decrease of microorganism count, depending on the type of microorganism and duration of exposure. The inactivation rate was higher for bacteria. In the case of yeasts the lethality rate was 55% for a 5 minutes UV treatment. Under the same conditions, lactic bacteria were inactivated at a rate of 59%
and acetic bacteria at a 62% rate. The increase of UV treatment duration to 10 minutes did not significantly increase microorganism inactivation rate (Table 1; Figure 1a).

Table 1. Influence of UV treatment on microorganism viability.

| Type of microorganism | V1 – 2 min. | V2 – 5 min. | V3 – 10 min. |
|-----------------------|-------------|-------------|--------------|
| Yeasts                | Viability (%) | 47 | 45 | 47 |
|                       | Lethality (%) | 53 | 55 | 53 |
| Lactic bacteria       | Viability (%) | 64 | 41 | 44 |
|                       | Lethality (%) | 36 | 59 | 56 |
| Acetic bacteria       | Viability (%) | 54 | 38 | 38 |
|                       | Lethality (%) | 46 | 62 | 62 |

Figure 1. (a) Influence of UV treatment on microorganism viability: 1) 2min; 2) 5min; 3) 10min; (b) Influence of the duration of application of pulsed electrical field (PEF) on microorganism inactivation: 1) Yeast; 2) Lactic bacteria; 3) Acetic bacteria;

Simple PEF treatment reduced microbe load in suspension, but the reduction rate was different depending on the type of microorganism and the duration of the treatment (Figure 1b). First degree correlations were established between the microbe load (expressed in CFU/ml) of microorganism groups (yeasts, lactic and acetic bacteria) and PEF treatment duration. Only regression equation parameters differ (Table 2).

Table 2. Correlation between microbial load and PEF treatment duration.

| Type of microorganism | Form of regression line | Regression coefficient |
|-----------------------|-------------------------|------------------------|
| Yeasts                | $y = 17.181x + 35.951$ | 0.9338                 |
| Lactic bacteria       | $y = 20.471x + 37.198$ | 0.9115                 |
| Acetic bacteria       | $y = 21.382x + 35.417$ | 0.8829                 |

Table 3. Influence of UV + PEF treatment on microorganism viability.

| Type of microorganism | UV 5 min | PEF 2 min | UV+PEF (5min + 2min) |
|-----------------------|----------|-----------|----------------------|
| Yeasts                | Viability (%) | 48.5 | 22.0 | 4.5 |
|                       | Lethality (%) | 51.5 | 78.0 | 95.5 |
| Lactic bacteria       | Viability (%) | 39.0 | 9.1  | 2.8 |
|                       | Lethality (%) | 61.0 | 90.9 | 97.2 |
| Acetic bacteria       | Viability (%) | 37.6 | 6.1  | 2.1 |
|                       | Lethality (%) | 62.4 | 93.9 | 97.9 |
The combined treatments UV + PEF (Table 3 and Figure 2a) and PEF + UV (Table 4 and Figure 2b) proved to be very efficient leading to an almost complete inactivation of microorganisms. The inactivation rate was higher in the case of PEF + UV treatment, for both lactic and acetic bacteria. Yeasts were more treatment resistant, with a 96% lethality rate under both treatments conditions (Table 4 and Figure 2b).

Figure 2. (a) Influence of UV + PEF treatment on microorganism viability: 1) UV 5min; 2) PEF 2min; 3) UV+PEF (5min+2min); (b) Influence of PEF + UV treatment on microorganism viability: 1) PEF 1min; 2) PEF 2min; 3) PEF 3min; 4) PEF+UV (2min+5min);

Table 4. Influence of PEF + UV treatment on microorganism viability.

| Type of microorganism | PEF 1 min | PEF 2 min | PEF 3 min | PEF + UV (2min+5min) |
|-----------------------|-----------|-----------|-----------|----------------------|
| Yeasts                | Viability (%) | 49.5      | 24.4      | 15.1                | 3.9                     |
|                       | Lethality (%)   | 50.5      | 75.6      | 84.9                | 96.1                    |
| Lactic bacteria       | Viability (%) | 46.0      | 14.5      | 5.1                 | 0.4                     |
|                       | Lethality (%)   | 54.0      | 85.5      | 94.9                | 99.6                    |
| Acetic bacteria       | Viability (%) | 47.7      | 12.8      | 4.9                 | 1.7                     |
|                       | Lethality (%)   | 52.3      | 87.2      | 95.1                | 98.3                    |

4. Conclusions
The paper presents a combined system for microbial inactivation of microorganisms (yeasts, lactic bacteria, acetic bacteria, in different concentrations in distilled water) involved in the processing of grape products. The results confirm PEF + UV system superiority over the PEF alone treatment system. UV radiation treatment is used only in PEF + UV combination when applying the Hurdle strategy. In conclusion, both non-thermal PEF and UV treatments, simple or combined, are promising for the field of enology, as they are inexpensive and have the ability to replace thermal treatments which have negative effects on product quality. However, combined treatments improve system performance and are therefore more efficient.

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