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Pulsed high pressure treatment for the inactivation of *Saccharomyces cerevisiae*: The effect of process parameters

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Abstract

This paper aims to investigate the effect of main processing variables on the inactivation rates of *Saccharomyces cerevisiae* cells suspended in commercial fruit juices and processed in pulsed high pressure treatments. The investigated variables were: pressure level, number of pressurization cycles, pulse duration and ramp rate. According to our experimental results, the efficiency of pulsed high pressure processes depends on the combination of pulse holding time and number of pulses. Pulsed high pressure is more effective than isostatic pressure, provided that the pulse holding time is higher than a threshold value. Moreover, no additive effect of the pulses could be detected, being the lethality of the single pulses reduced when increasing the number of pulse and the ramp rate. These findings are confirmed at different pressure levels with all processing media utilized. If several pressurization cycles with a defined number of pulses are applied, the faster the compression rate the lower is the final inactivation level achieved.
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Keywords: Pulsed high pressure; Fruit juice; Ramp rate; Pulse duration

1. Introduction

The utilization of mild technologies in the microbial stabilization of foods is gaining more and more interest, due to the increasing consumer's inclination toward fresh-like minimally processed products, which are considered more safe and healthy than thermally processed products. This has recently stimulated the development of non-thermal techniques for food preservation, designed to prevent the degradation of thermosensitive components, which are mainly responsible for aroma characteristics and for most peculiar nutritional properties of food (like antioxidant power, vitamin content, etc.) strictly connected with the image of freshness perceived by consumers.

Among other mild preservation techniques, the sanitization of foods from microbial spoilage by means of very high hydrostatic pressures has been proposed in the last twenty years as a low-impact non-thermal technique to be used in the manufacturing of high quality products, foods and food ingredients, and has been intensively studied all over the world. In spite of the intrinsic validity of the high pressure process and of the large amount of scientific work performed on the subject, relatively few commercial applications are reported and, moreover, all of that are set up for high added value products. This is mainly due to the high fixed cost of the pressurization apparatus and to the necessity of batchwise operation for most of the processes envisaged. Considering that the complexity and the cost of pressure equipment rise more than linearly with the maximum operating pressure, the main requirement to make this process economically sustainable is the reduction of the pressure level necessary to attain a commercially suitable microbial inactivation level on the food processed.

The problem of finding new tools for lowering the peak pressure needed in high pressure sanitization has been recently faced by scientists according to different strategies,

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all directed to reduce the maximum pressure level of the process by synergistic effects. The first approach is directed toward the coupling of the effect of other physical agents, like temperature or pH, with high pressure, to improve the inactivation rate of microorganisms at a given pressure, according to the so called "hurdle technologies" or "combined treatment cycle" principle. Some valuable results, obtained in particular by simultaneous or sequential application of pressure and temperature, demonstrated that this approach based on synergetic effects can be considered promising in industrial food processing (Donsì, Ferrari, & Maresca, 2003, 2005). The second approach is directed to try either to add dynamic components to the static pressure distribution obtained in hydrostatic pressurization or to apply repeated pressure pulses to the sample to be treated, in the view that dynamic effects may enhance the inactivation rate of microorganisms obtained under static conditions. At the moment the potential benefits of these techniques are yet matter of concern, even if some promising results are reported in literature (Aleman et al., 1996; Ardia, Heinz, & Knorr, 2004; Noma, Tomita, Shimoda, & Hayakawa, 2004). The application of dynamic or repeated pressure pulses requires more sophisticated apparatus, able to stand fast pressure rise or decay transients and to apply repeated sequences of pressure cycles. The availability of new technologies for pressurization, directed to eliminate or to speed up the operation of pressure intensifiers, which are an intrinsically slow piece of equipment and cannot produce repeated pressurization cycles, enhances the interest for a deeper investigation on the use of pulsed pressure treatments.

This paper deals with an experimental investigation on the use of pulsed pressure treatment for the microbial sanitization of fruit juices. To get free of complex effects deriving from the different nature and growing stage of microorganisms in the original juice, samples treated are artificially inoculated with a specific strain of *Saccharomyces cerevisiae*, grown up to a defined concentration. The effect of the maximum pressure level attained, pulse number and duration, pressurization rate and treatment temperature is analysed for samples of orange and pineapple juice. Results are analysed and the surviving fraction of yeast is determined. Data are worked out to suggest a strategy for studying the optimal combination and characteristics of pressure pulses to obtain commercial sanitization of juices with the minimum cost.

2. Materials and methods

2.1. High pressure multi-vessel system U111

The apparatus used for the experiments is a high pressure multi-vessel system (U111 apparatus by Unipress, Poland). This system can operate at pressures up to 700 MPa and temperatures from −40 to 100 °C. The main components of the apparatus, sketched in Fig. 1, are the following:

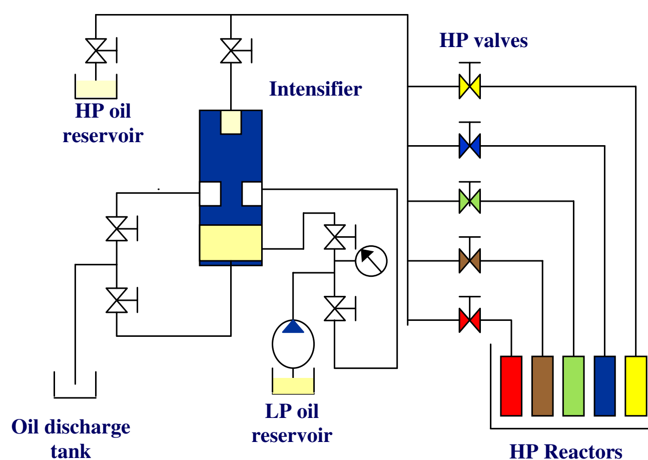

Fig. 1. Schematic view of the high pressure multi-vessel U111.

- five high pressure reactors, made out of Cu–Be alloy, working in parallel, each with an internal volume of 9 ml. A screwed plug closes the reactors and can be manually opened to introduce the samples. Each reactor is equipped with a manual cut-off valve and thus, even if fed by a single same pressurization device, can operate at different pressure levels for different holding times;
- the pressurization system consisting of a hydraulic low-pressure pump connected to a high pressure intensifier;
- the pressure release system consisting of a high pressure manual valve;
- the control unit which has the following functions: set up of the pressure level, set and control of the pressurization ramp, start of the filling of the intensifier and of the reactors, measurement and control of pressure and temperature levels in the reactors;
- the data acquisition system U111-DAS;
- the heating–cooling unit, consisting of a thermostatic bath containing the five reactors.

2.2. Preparation of the samples

S. cerevisiae yeast was grown in MRS broth (OXOID) at 32 °C in an aerated incubator (Haeraeus Instruments). Samples were taken after 40 h, to be sure that the stationary growth phase was attained. To perform each test, three samples of 10 ml of yeast cells in MRS broth were centrifuged at 5000 rpm and 20 °C for 5 min. The microbial pellet obtained was suspended in a volume of commercial fruit juice and diluted to reach a final microbial concentration of 10^6 cfu/ml. Samples were sealed in flexible pouches made of coupled polymer and aluminium film (PE–Al). Pouches were introduced in the high pressure reactors and the holding time was counted after the build up of the selected pressure level. To avoid the modification of cell concentration by natural growth after processing, pouches after the treatment were stored at 4 °C before the microbiological assay. Each experiment was replicated at least three times.

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