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REVIEW

Inactivation of *Saccharomyces cerevisiae* using new non-thermal technologies. A review

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Abstract

This paper aims to review the available literature data and provide a general view of the application of several new non-thermal treatments: high pressure (HP), pulsed electric fields (PEF) and pulsed light (PL) on the inactivation of S. cerevisiae as spoilage yeast. Largely investigated at present, these technologies proved to be promising alternative to thermal processing of food. Several investigations on S. cerevisiae inactivation are discussed for each of these new techniques, together with data about the amount of input energy. The review demonstrates that HP, PEF and PL are able to inactivate S. cerevisiae as spoilage yeast in food.

Keywords: spoilage yeast, high pressure, pulsed electric field, pulsed light.

Introduction

Saccharomyces cerevisiae is a yeast that have been known to humans for thousands of years as it has been used in fermentation processes like the dough leavening, winemaking and brewing. In nature, yeast cells are found primarily on the skin of ripe fruits such as grapes (BOULTON & QUINN [1]; FELDMANN [2]). *S. cereveisiae* is not airborne; therefore it requires a vector to move. STEFANINI et al. [3] showed that queens of social wasps surviving the winter as adults (*Vespa crabro* and *Polistes* spp.) can harbour yeast cells into their gut from the end of summer to spring and transmit them to the new generation of larvae. *S. cerevisiae* is a unicellular microorganism having round to ovoid cells, 5-10 micrometres in diameter which reproduce by budding. It can survive and grow either in haploid form in which cells undergo a simple mitosis and grow, or in diploid form which is preferential. In this last form cells similarly undergo a simple life cycle of mitosis and grow, but do no die under conditions of stress since they can undergo sporulation through meiosis, producing four haploid spores, which can proceed on to mate (HERSKOWITZ [4]).

Besides its use in various fermentation processes, some strains of *Saccharomyces* spp. are frequently involved in food spoilage. Thus, they can spoil wines, beers and other alcoholic beverages by producing gassiness, turbidity and off-flavours associated with acetic acid and hydrogen sulphide (LOUREIRO & MALFEITO-FERREIRA [5]). Some species grow on fresh fruits and vegetables such as grapes (LOUREIRO & MALFEITO-FERREIRA [5]) and bean sprouts (RASCH et al. [6]), or on processed fruits such as fruit juices (SIEGMUND & POLLINGER-ZIERLER [7]) or fruits added to yoghurt (VILJOEN et al. [8]).

Fruit juices generally have relatively high levels of carbohydrates and a low pH which favours the growth of spoilage yeasts, moulds and some acid-tolerant bacteria. Spoilage may be manifested as surface pellicles, fibrous maths, cloudiness and off-flavours. *Saccharomyces*

spp. resistant to thermal processing is found in several spoiled juices (FITZGERALD et al. [9]; SILVA & GIBBS [10]).

Currently, the heat treatment is still the most common, available and best understood method of inactivation of microorganisms and enzymes, thereby prolonging the shelf life of food. With regard to the food safety, the stabilization of food to the action of yeast, hence the *S. cerevisiae* is carried out by pasteurisation, meaning that it is not necessarily a powerful treatment as sterilisation. Table 1 presents *D*- and *z*-values obtained for *S. cerevisiae* yeast either as vegetative spores or as ascospores after heat treatment of different media.

Table 1. *D*- and *z*-values of *S. cerevisiae* for thermal treatment

Cell condition	Medium	pH	*D*, min	Temp., °C	*z*, °C	References
Ascospores	Apple juice 8.6 °Bx	3.6	6.10	60	3.8	SPLITTSTOESSER et al. [11]
Ascospores	White wine, Chenin blanc, 0.5 °Bx	3.1	4.80	48	6.7	SPLITTSTOESSER et al. [11]
Vegetative cells	Citrate buffer	4.0	2.80	60	3.5	SHEARER et al. [12]
Vegetative cells	Apple juice 11.7 °Bx	3.5	13.00	57	6.0	SHEARER et al. [12]
Vegetative cells	Apple juice 11.7 °Bx	3.9	9.10	57	4.0	SHEARER et al. [12]
Vegetative cells	Apple juice modified (Ca fortified)	3.9	32.00 6.90 2.10	57 60 63	5.1	SHEARER et al. [12]
Vegetative cells	Grapefruit juice 10.4 °Bx	3.3	9.30 2.80 0.98	57 60 63	6.1	SHEARER et al. [12]
Vegetative cells	Mixed juice product 13.5 °Bx	2.8	9.40 2.80 0.40	57 60 63	5.8	SHEARER et al. [12]
Vegetative cells	Tomato juice	4.2	16.00 4.10 0.64	57 60 63	4.3	SHEARER et al. [12]
Ascospores	Citrate phosphate buffer	4.5	15.60	60	5.5	PUT & DE JONG [13]

Unfortunately, heat processing may cause significant changes in the quality and organoleptic characteristics of food. The increasing concern of the consumers for fresh-like, minimal processed food products and the negative attitude to the addition of chemicals in food has led to the development of alternative methods of processing. Non-thermal processing methods include:

- high hydrostatic pressure (HHP) or high pressure processing (HPP) of fruits, juices, meat and fish (TORRES & VELASQUEZ [14]; OEY et al. [15]);
- pulsed electric fields (PEF) of juices (EVRENDILEK et al. [16]; CHARLES-RODRÍGUEZ et al. [17]);
- ultraviolet (UV) light treatment (BINTSIS et al. [18]; KOUTCHMA [19]; GABRIEL [20]);
- pulsed light (PL) treatment (KOUTCHMA et al. [21]);
- ultrasound (US) technology (RASTOGI [22]).

Most of these non-thermal new techniques were successfully applied for inactivation or decontamination on different food.

The aim of this paper is to review the available literature data and provide a general view of the application of some new non-thermal treatments (HP, PEF and PL), on the inactivation of *S. cerevisiae* as spoilage microorganism in food.

Inactivation of *Saccharomyces cerevisiae* using high pressure processing

High pressure processing (HPP) is a method of preserving and sterilizing food, in which a product is processed under very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food. The effect of high pressure on microorganisms and proteins/enzymes was observed to be similar to that of high temperature. HPP stops biochemical activity caused by microorganisms that play a role in the deterioration of foods. It does not include the use of food additives. The treatment occurs at 100 to 600 MPa, for 1-15 min, with the initial product temperature of 5...25°C, which is called high pressure pasteurisation (HP-P) and inactivates vegetative pathogens. Another category of HPP is high pressure sterilisation (HP-S), or HPHT (high pressure, high temperature) which occurs al 400-700 MPa, with the initial product temperature of 70...90°C, the process temperature of 110...120°C and the holding time of 1 to 10 min. This method also inactivates bacterial spores (GRAUWET et al. [23], RASTOGI et al. [24]).

Although that killing of microorganisms when applying high pressure was discovered way back in 1899 and has been used in different fields, HPP became available to the food processing industry late in 1980's (RASTOGI et al. [24]).

Several studies concerned on the inactivation of *S. cerevisiae* with HPP exist in literature among which are those written by CHEN & TSENG [25]; BASAK et al. [26]; PERRIER-CORNET et al. [27]; BUZRUL [28].

CHEN & TSENG [25] combined different hydrostatic pressures (0.1-300 MPa) and temperatures (35...55°C) and determined the inactivation kinetics of *S. cerevisiae* CCRC 20271. The average activation energy (E_a) was $1.65 \pm 0.61 \times 10^4$ J/mol for 0.1-100 MPa and $1.23 \pm 0.17 \times 10^5$ J/mol at 150-300 MPa. Therefore, the combined effect of pressure and temperature on the survival of the yeasts tended to increase stepwise as the pressure increased.

Further investigations were performed in single strength and concentrated orange juice at 100-400 MPa and holding times (0-120 min) at 25°C (BASAK et al. [26]). The pressure dependency of kinetic parameters was well described by the z_p values (pressure range to result in a decimal change in D values) of 135 MPa in the single strength orange juice and 287 MPa in the concentrate.

PERRIER-CORNET et al. [27] pressurized *S. cerevisiae* for 10 min at temperatures between –20 and 25°C and pressure between 100 and 350 MPa. The effect of pressure was significantly enhanced applying high pressure at negative temperatures without freezing. An increase in the pressure-induced inactivation from about 1-log up to 7-8-log was obtained decreasing the temperature from ambient to – 20°C at a pressure of 150 MPa.

In addition to microbial destruction, BUZRUL [28] showed that HHP improves some organoleptic properties of beer and wine without detrimental effects on important quality characteristics, such as colour, pH and turbidity.

To sum up, the HPP treatments presented in these examples occur at lower temperatures than thermal pasteurisation so that food were subjected to less thermal stress and sensory qualities were better preserved. Another advantage of HPP methods is that they do not include the use of food additives.

HPP is an alternative technology to heat treatment that has reached consumer market with a large variety of products made of vegetables or raw fruit materials, such as juices, jams, jellies, fruit or veggie sauces, avocado pulp, guacamole, etc. (LÓPEZ-FANDIÑO, [29]).

Inactivation of *Saccharomyces cerevisiae* using Pulsed Electric Fields

The concept of PEF was first proposed in 1967 and consists in application of high voltage electric field at a certain level for a very short time (RAVISHANKAR et al. [30]). When applied to food, this non-thermal processing technology can have a lethal effect to microorganisms. The direct cause of cell inactivation is the membrane damage under PEF action. The electric field enlarges the pores of the cell membranes until they break down. If the electrical field strength exceeds the critical value for a given duration, permanent holes are formed which kills the cells and releases their contents (ARONSSON et al. [31]).

The lethal effect of PEF to microorganisms was studied in 1990s to provide consumers with microbiologically-safe and fresh-like quality foods. PEF not only inactivates pathogenic and spoilage microorganisms, but also results in the retention of flavour, aroma, nutrients and colour of foods. PEF technology has limited industrial application for the pasteurization of fruit juices. The first commercial PEF pasteurization of apple cider product took place in the United States in 2005 (RAVISHANKAR et al. [30]).

The literature presents a broad variety of investigations concerning the effect of PEF treatment on *S. cerevisiae* pursuing the process parameters for cells inactivation, changes in the cell membrane structures, factors influencing the inactivation and sensory characteristics evolution after PEF treatment. A selection of the most important findings is presented below.

ARONSSON et al. [31] examined the killing effect of PEF on *S. cerevisiae* using electric field strength of 25-35 kV/cm and 20-40 pulses of 2-4 μs pulse duration. They achieved a reduction of 6-log when exposing the cells to 30 kV/cm and 4 μs pulse duration, irrespective of the number of applied pulses.

Membrane permeabilization of *S. cerevisiae* cells caused by PEF treatment was further investigated by measurement of propidium iodide (PI) intake (ARONSSON et al. [32]). Inactivation of cells was determined by viable counts, and leakage of intracellular compounds, such as ATP, was measured in the extracellular environment. Membrane permeabilization of *S. cerevisiae* was influenced by electrical field strength (5 to 30 kV/cm) and pulse duration (2 and 4 μs), the increase of these parameters resulting in higher PI uptake and larger amounts of intracellular compounds leaking from cells, therefore in enhanced inactivation. However, cells of *S. cerevisiae* were not necessarily lethally permeabilized, meaning that higher parameters of PEF treatments than those used in this study would be required to destroy the cells of *S. cerevisiae*.

CZERHALMI et al. [33] investigated the effect of PEF treatment, applied in a continuous system, on *S. cerevisiae* cells inoculated into sterilised apple juice. The reduction of *S. cerevisiae* cells was approximately 4-log when 10.4 pulses at 20 kV/cm were used. The study showed that the inactivation of *S. cerevisiae* depended on the electric field intensity and number of pulses, increasing when these were enhanced.

Electric field strength, cumulated treatment time and initial cells concentration were investigated for the microbial inactivation of *S. cerevisiae* inoculated in liquid and solid model foods and treated with PEF in a batch chamber (DONSÌ et al. [34]). The maximum level of inactivation of achieved for cells suspended in liquid was 4.51-log CFU/mL at 30.9 kV/cm electric field strength after a total process time of 1600 μs. The effectiveness of microbial inactivation process enhanced with agitation of liquid samples, e.g. the inactivation level increases from 3.05 to 5.93-log at a treatment time of 200 μs at 22.6 kV/cm if the liquid sample is stirred every 8 pulses. Experiments carried out immobilizing the *S. cerevisiae* cells in potato dextrose agar, a solid model food, confirmed the heterogeneous distribution of the electric field in the treatment chamber. The effect of initial inoculation level on the effectiveness of PEF was also studied and it is still contradictory. Tests of Donsì et al. [34])

with inocula in the range of 10^3-10^8 CFU/mL showed that the level of inactivation achieved increases with decreasing the initial concentration of *S. cerevisiae* cells after PEF treatment.

EL ZAKHEM et al. [35] investigated the effect of PEF to colloidal suspension of *S. cerevisiae*. They measured the electrical conductivity during PEF treatment in the range of 3-15 kV/cm for 10^{-4} to 1 s. The results confirmed that electric field strength of 7.5 kV/cm is a threshold value for *S. cerevisiae* culture. Thus, the damage of cells below this value was incomplete and developed at long time of PEF treatment. The lethality of cells induced by PEF enhanced with the mixing of suspensions and the increase of temperature.

HUANG et al. [36] applied PEF technology to control the microbiological spoilage of Chinese rice wine and studied the effects of electric field intensity, treatment time and initial temperature. The spoilage yeast commonly associated with rice wine is *S. cerevisiae*. Applying a range of electric field strength of 12-21 kV/cm for 30-180 μs, the destruction of cell membrane structures was induced. The highest inactivation value of approximately 5.5-log was obtained at 21 kV/cm and 180 μs with the initial treatment temperature of 30 and 35°C. The effects of PEF treatment on concentration in °Brix, pH, conductivity and colour of rice wine immediately after the treatment and during storage at 22°C were also investigated. The results indicated that PEF treatment was able to inactivate *S. cerevisiae* in rice wine with minor changes in the physicochemical parameters (Huang et al. [36]).

During the last two decades, PEF started to have industrial applications for sterilisation in food processing such as orange juice, sugar beat, and mushrooms, besides water decontamination, medical treatments and material processing (AKIYAMA et al. [37].

Inactivation of *Saccharomyces cerevisiae* using Pulsed Light treatments

Pulsed light (PL) is an emergent non-thermal technology investigated as a promising alternative to traditional thermal treatment for inactivating spoilage and pathogenic microorganisms on food and food contact surfaces (GÓMEZ-LÓPEZ et al. [38]). PL treatment involves the use of a clear fused quarts tube filled with xenon, which does not contain mercury as UV lamps (US FDA/CFSAN [39]). This lamp is able to produce short pulses of intense broad-spectrum of electromagnetic radiation (BARBOSA-CÁNOVAS et al. [40]; ELMNASSER et al. [41]; OMS-OLIU et al. [42]). According to DUNN et al. [43], this spectrum is very similar to sunlight and contains wavelengths from the ultraviolet (UV) domain (200 nm) to the near-infrared region (1000 nm), having peak emission between 400 and 500 nm. Therefore, pulsed light represents an improved version of UV treatment (BINTSIS et al. [18]).

The ability of PL to inactivate *S. cerevisiae* as spoilage microorganism has been demonstrated in several studies (ROWAN et al. [44]; TAKESHITA et al. [45]; FINE and GERVAIS [46]; KAACK & LYAGER [47]; AGUILÓ-AGUAYO et al. [48]; FERRAIO et al. [49]).

ROWAN et al. [44] investigated the effect of PL on the survival of predetermined populations of several bacterial cultures and yeast *S. cerevisiae*. *S. cerevisiae* was grown in malt extract broth at 25°C to a cell density of about 10^9 cells/mL, than subjected to PL treatment. The experimental set-up was equipped with two xenon flash lamps. One lamp was of clear fused quartz-tube transparent to UV and the other one with a cerium-doped quartz envelope which restricted the light output in the UV region. The inactivation of *S. cerevisiae* was 4.9-log CFU/plate for PL with high UV light and only 0.7-log CFU/plate for PL with low UV light when 200 pulses were applied. The results showed that significantly greater levels of *S. cerevisiae* cells inactivation ($P < 0.001$) occurred with PL of high UV content (ROWAN et al. [44]).

In 2003 WUYTACK et al. [50] concluded that pulsed white light inactivation should be regarded as a multi-target process. In this, structural changes to DNA would be a major reason, and damage to membranes, proteins and other macromolecules plays a minor role (WUYTACK et al. [50]; GÓMEZ-LÓPEZ et al. [38]). The hypothesis is in line with the results of TAKESHITA et al. [45], who compared the inactivation of *S. cerevisiae* by continuous UV and broad-spectrum PL. In their work, cells viability was reduced from 7×10^6 CFU/ml to 10 CFU/ml after five pulses at 0.7 J/cm^2/pulse of PL treatment (total: 3.5 J/cm^2), that meaning a reduction of about 5.8-log. The results showed that both light sources induced DNA damage such as the formation of single strand breaks and pyrimidine dimmers in yeast cells. However, protein elution was higher in pulsed than in continuous UV treatment. This suggested cell membrane damage induced by pulsed light. Expanded vacuoles and distorted membranes were detected in 50% cells after only three light flashes, but were hardly found under continuous UV treatment (TAKESHITA et al. [45]).

FINE & GERVAIS [46] achieved a 7-log reduction of *S. cerevisiae* dried in glass beads and quartz plates, 2.93-log reduction on black pepper subjected to a dose of 31.12 J/cm^2, and a 0.7-log reduction on wheat flour. However, regarding results of experiments with microorganisms spread on agar surfaces, in the incubation method of survivors counting, GÓMEZ-LÓPEZ et al. [51] warned about a potential overestimation of the lethality due to the possibility that two or more surviving microorganisms situated very close to each other can form a single colony.

In other study, carrot sliced were inoculated with *S. cerevisiae* and treated with PL from two flash lamps, located below and above the samples (KAACK & LYAGER [47]). The authors found that the major part of the yeast cells were destroyed using two pulses, with each pulse delivering 0.7 J/cm^2, and the reduction was of 3.07-log CFU/g. After exposure to 24 pulses, reduction was 4.93-log CFU/g. The authors concluded that, in combination with washing, PL may reduce the level of yeast cells by up to 6-log cycles.

AGUILÓ-AGUAYO et al. [48] investigated the effect of PL on surface decontamination, physical properties (colour, texture and weight) and nutritional quality (ascorbic acid and major carotenoids) in red-ripe tomatoes during 15 days of storage at 20°C. PL treatments at fluences of 2.68 and 5.36 J/cm^2 reduced the microbial loads during storage of whole tomatoes. One log reduction of natural microbiota present on tomato skin was obtained with a fluence of 4 J/cm^2. PL treatments with fluences of 2.2 J/cm^2 allowed a 2.3-log reduction of *S. cerevisiae* inoculated on the tomato surface. Ascorbic acid was not affected by PL treatment. Tomato extract obtained from fresh tomato fruits treated with a 30 J/cm^2 PL dose had higher lycopene, α-carotene and β-carotene contents. The authors concluded that PL treatment of fresh tomato reduced the microbial contaminants without compromising the nutritional value and induced some appearance defects.

FERRAIO et al. [49] investigated the inactivation of *S. cerevisiae* KE162 in commercial juices and fresh squeezed juices. They found a negative relationship between the turbidity of the juices with pulp and PL effectiveness since pulses were not able to penetrate the cloudy juice and reach the cells of the yeast. It is well known that PL act on the surfaces (LASAGABASTER et al. [52]) and in thin layers of clear liquids (SAUER and MORARU [53]; PATARO et al. [54]). Thus, PL treatment at 2.4-71.6 J/cm^2 was ineffective in fresh strawberry and orange juice. However, PL treatment proved to be efficient in fruit juices such as apple and melon juices (FERRAIO et al. [49]).

In 2005, the Food and Drug Administration Department of Health and Human Services approved the use of PL technology for production, processing and handling of foods in a regulation on food irradiation (U.S. FDA 2005 [55]). According to this regulation, PL treatment is applied to control the microbial load on surfaces and the total cumulative treatment of PL shall not exceed 12.00 J/cm^2.

Conclusions

This review showed that high pressure (HP), pulsed electric fields (PEF) and pulsed light (PL) have powerful effects on the inactivation of *S. cerevisiae* as spoilage yeast in food. These technologies belong to the new non-thermal technologies largely investigated at present as alternative methods to thermal processing of foods. They have proven positive action in the inactivation of spoilage yeast, group to which *S. cerevisiae* belongs, in particular and of spoilage and pathogen microorganisms in general. Using these technologies, fresh-like, minimal processed food with no addition of additives could be supplied to consumers. Moreover, flavour compounds, vitamins and other nutrients are best retained in food and sensory characteristics are little or not at all affected during processing with HP, PEF or PL. Most of reviewed studies provide information about the amount of energy needed in treatment, so their comparison is possible. Therefore, HP, PEF and PL are promising technologies to satisfy modern trends in food consumption.

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