# Investigation of the effect of water exposed to nonequilibrium contact plasma onto saccharomyces cerevisiae yeast

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## Abstract

**Introduction.** Additional treatment of water by nonequilibrium contact plasma allows improving consumer characteristics of bakery goods considerably. Determination of the effect of plasma-chemically activated water on morphological, cultural and physiological properties of Saccharomyces cerevisiae yeast is important from the technological point of view.

**Materials and Methods.** Experimental investigations were carried out in the conditions of bacteriological laboratory by seeding the culture of yeasts of TM "Lvivski" and "Kryvorizki" on Sabouraud dense liquid nutrient media. The quantity of viable cells of microorganisms was determined by the method of Gould sector seeds. Morphology of the yeast was investigated by phase-contrast microscopy. Biotechnological properties of yeasts were determined on Giss media.

**Results.** The paper establishes the effect of water exposed to nonequilibrium contact plasma on the sensitivity of Saccharomyces cerevisiae and shows absence of suppressive action of treated water with regard to cultural properties of microorganisms. The experiments prove that with the use of plasma-chemically activated water morphological characteristics and biochemical properties of bakery yeasts produced by Lviv and Kryvyi Rig yeast plants are preserved. Culturing of Saccharomyces cerevisiae yeast on the nutrient media prepared with the use of water exposed to nonequilibrium contact plasm resulted in 6,5–15 times' increase in quantity of viable microorganisms compared with the control on the mains drinking water.

**Conclusions.** Physiological properties of Saccharomyces cerevisiae yeast improved owing to use water exposed to nonequilibrium contact plasma. Results of investigations are recommended for using in yeast production and bread making.

## Introduction

Food and processing industry play a key role in the national economy of Ukraine, since the citizens' health directly depends on high-quality nutrition. According to global food pattern indices, share of cereals and bakery products amounts to 51 % of total foodstuffs consumed by population of the globe. In particular, the dominating role in present-day diet of Ukrainians is traditionally given to bakery products. As the most of foodstuffs, during the manufacture of which structural biopolymers undergo significant transformation of properties, substantial amount of breadstuffs cannot do without using additives of artificial origin. However, over recent years the number of consumers caring of their health is growing, so the market of environmentally safe production where the usage of improvers is limited features increasing opportunities for expansion in Ukraine.

The leading place in food industry is occupied by biotechnology where microorganisms are dominating initiators of transformations. Microbiological and biochemical processes occurring in the source raw materials throughout the technological process make a significant contribution into formation of organoleptic and physical-chemical properties of finished products. Microbiological processes running in heterogeneous food systems have a number of peculiar features connected with their component composition which may vary considerably.

Water is one of the basic raw materials in bread production. Most often, drinking water without any treatment is used in the process, but it is reported that in case of additional treatment thereof, for example, by plasma-chemical activation, it is possible to improve the technology of bakery products considerably [1]. It is found that owing to usage of plasma-chemically activated water instead of mains drinking water without additional treatment the dough maturation is accelerated by 20 % on average, compared with the control. Besides, technological characteristics of yeast, namely, fermentation property and osmo-sensitivity thereof, improve by 10–15 % and 40–45 % accordingly [2, 3]. However, the papers mentioned above do not deal with the action of water exposed to nonequilibrium contact plasma (NCP) on the bakery yeast beyond the heterogeneous food systems. Since the yeast is the main driving force of microbiological processes which occur during maturation in wheat semi-finished products, the purpose of this paper consists in investigation of the effect of plasma-chemically activated water on sensitivity, morphology and cultural properties of Saccharomyces cerevisiae.

## Materials and methods

Pressed bakery yeast Saccharomyces cerevisiae is the main object of investigations. The work deals with the yeasts of trade marks (TM) "Lvivski" produced by Lviv yeast plant (Enzym PJSC) and "Kryvorizki" produced by Kryvyi Rig yeast plant ("Nadezhda" CJSC) as those being in good demand of Ukrainian bakery enterprises. It should be noted that each enterprise produces the yeasts following its own technical specifications stating the better quality indices and longer term of storage compared with the yeasts produced following national quality standard [4]. Strains of yeast used in production are not disclosed by the manufacturers for obvious reasons.

Investigators used mains water without additional treatment and water exposed to NCP, with the characteristics given in Table 1. Treatment of water with nonequilibrium contact plasma was carried out in the laboratory of plasma-chemical technologies of the Public higher education institution "Ukrainian State University of Chemical Technology" at the

discrete-type laboratory unit with the reactor volume of 0,1 dm3 (Fig. 1). For preparing control samples, drinking water of the Dnipropetrovsk city mains was used.

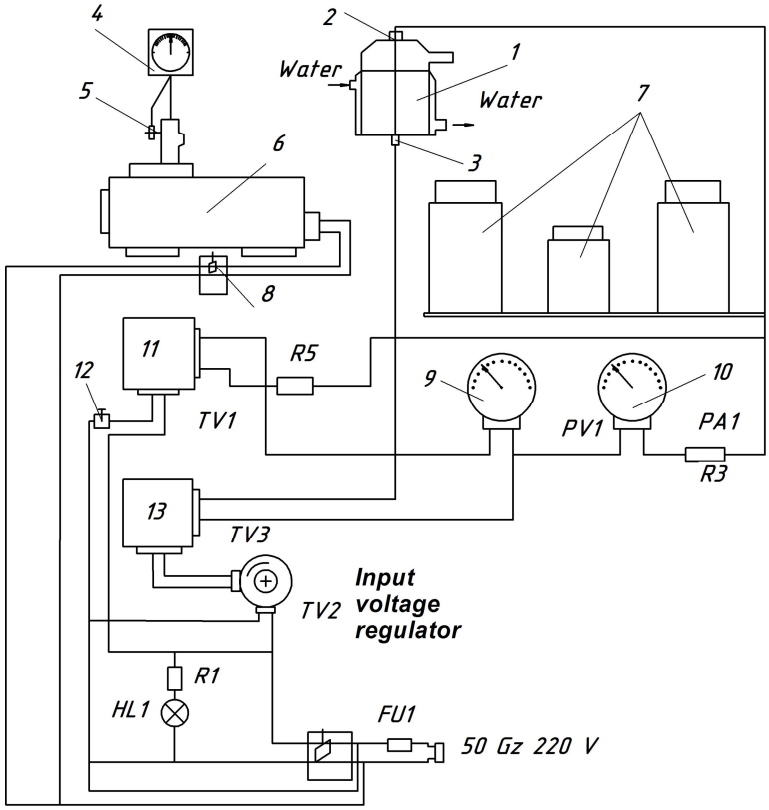


**Fig. 1. Apparatus for plasma-chemical treatment of water:**
1 – reactor; 2, 3 – electrodes; 4 – vacuum gage; 5 – cock; 6 – pump; 7 – filtering elements; 8 – switch; 9 – voltmeter; 10 – ammeter; 11 – ignition transformer; 12 – switch; 13 – voltage transformer

In order to determine sensitivity of yeast to water exposed to NCP, yeasts produced by Lviv and Kryvyi Rig yeast plants were cultured on Sabouraud medium for 24 hours at temperature of 28°C. After that 0,1 ml of the microbial suspension of 1 bln/ml dilution were seeded with a lawn on Sabouraud agar with 20 ml of agar per one Petri dish. Dishes with seeds were dried, and after that four wells of 5 mm in diameter were formed in agar. Obtained wells were filled with 0,1 ml of water exposed to NCP with various characteristics (Table 1). After incubation of seeds during 24 hours at 28 °C the degree of delay in growth of the culture in millimeters was determined.

**Table 1**

**Characteristics of mains drinking water and plasma-chemically activated water**

| No. of sample | | Duration of NCP action on water, *minutes* | Concentration of peroxide compounds, *mg/l* | pH of water | Oxidation-reduction potential, *mV* |
|---|---|---|---|---|---|
| 1 | Mains water without additional treatment | – | – | 7,0 | 240 |
| 2 | Plasma-chemically activated mains water | 3 | 100 | 10,1 | 123 |
| 3 | Plasma-chemically activated mains water | 7 | 300 | 10,3 | 110 |
| 4 | Plasma-chemically activated mains water | 9 | 500 | 10,4 | 95 |
| 5 | Plasma-chemically activated mains water | 12 | 700 | 10,2 | 90 |

For investigating the effect of water on the yeast morphology, pure daily culture of Saccharomyces cerevisiae was seeded with inoculating loop on liquid Sabouraud agar prepared with the use of mains water without additional treatment (control) and plasma-chemically activated water with 500 mg/l concentration of peroxide compounds (sample 4). Cultural properties of microorganisms were determined after incubation of seeds during 24 hours at 28 °C. Morphological peculiarities of yeast microorganisms were additionally studied using the phase-contrast microscopy method. For that purpose, micro-chambers were prepared as follows: agar layer of 17×17×1 mm was placed onto preparation glass; 1 bln/ml suspension of Saccharomyces cerevisiae daily culture was applied onto its surface using the inoculating loop, with the cover glass put on top, and sealing of the sample with paraffin. Prepared micro-chambers were examined by the 100-power microscope.

Bakery yeasts cultured in the liquid medium were studied separately. Pure daily culture of yeasts was seeded in 5% Sabouraud broth prepared both on the mains water and plasma-chemically activated water. Seeds were incubated for 24 hours at temperature of 28°C; after that cultural properties of yeasts were determined. Quantitative content of viable cells of Saccharomyces cerevisiae in broth for experimental and control samples was determined by the method of Gould sector seeding on Sabouraud agar. Morphology of yeast cultured in Sabouraud broth prepared on the basis of mains water without additional treatment and plasma-chemically activated water was studied by means of phase-contrast microscopy. Biochemical properties of the grown Saccharomyces cerevisiae yeast were determined on Giss media. Bacteriological investigations were carried out following the standard practice (Klymniuk S.I., Sytnyk I.O., Tvorko M.S., Shyrobokov V.P. (2004), Praktychna mikrobiolohiia, Ukrmedknyha, Ternopil).

## Results and discussion

Testing of microorganisms for sensitivity to components of chemical or biological origin is of top priority from the practical point of view. In bakery production, yeast promotes the alcoholic fermentation in dough semi-product with the generation of secondary metabolites which form organoleptic properties (taste, flavor, coloring of the crust) and physical-chemical characteristics (porosity, acidity) of the finished products. Running of microbiological processes depends on a number of factors of different origin, among which a special role is given to stress factors as those resulting in various damages of the cell constituents and, as a consequence, even the cell death. For example, occurrence of oxidative stress is conditioned by active oxygen forms (AOF). Increase in protons' concentration often leads to suppression of many metabolic processes. Accumulation of anions in the cell under aerobic conditions can increase the intensity of free radicals' generation, that is, cause the oxidative stress [5], i.e. a set of response reactions aimed at overcoming adverse changes in the environment under the action of stress. Because of NCP action in the liquid, owing to processes of ionization, dissociation and dissociative attachment of plasma electrons to water molecules, thermal dissociation and dissociation through excited vibrational levels of water molecules in collisions $H_2O - H_2O$ the ions, atoms and molecules like $H_2O^+$, $OH^-$, $H^-$, $OH^-$, $H^+$, $H_2$, are formed, which initiate many chemical ion-molecular reactions and cause the accumulation of molecules of $H_2O_2$, $H_2O_3$, $H_2O_4$ (1–3) and other peroxide-type compounds (up to $H_2O_{10}$) in water:

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \quad (1)$$

$$HO_2^{\bullet} + OH^{\bullet} \rightarrow H_2O_3 \quad (2)$$

$$HO_2^{\bullet} + OH_2^{\bullet} \rightarrow H_2O_4 \quad (3)$$

After treatment with NCP, water acquires small-cluster structure, and its penetrating capacity increases which is shown by spectral and physical-chemical methods [6]. Therefore, it is important to ascertain whether the usage of plasma-chemically activated water would play the role of stress factor for Saccharomyces cerevisiae development. Regarding the other microbiological cultures, the paper [7] investigates the effect of plasma-chemically activated water and aqueous solutions on the pathogenic and opportunistic-pathogenic microorganisms, such as Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Escherichia coli, Pseudomonas aeruginosa, Candida ablicans, Streptococcus pyogenes, Proteus vulgaris, Klebsiella ozaenae. In particular, the action of chemical hydrogen peroxide on test cultures in concentrations approaching to the relevant values in plasma-chemically activated water was determined. Complete absence of antimicrobial action of hydrogen peroxide solutions of 10–100 mg/l concentration and poor sensitivity of microbiological cultures under study to the exciter of 500 mg/l concentration was shown. At the same time, test cultures demonstrated stable sensitivity to usage of plasma-chemically activated water with the concentration of peroxide compounds corresponding to similar hydrogen peroxide solutions (Table 2). It is found that the effect of water exposed to NCP on the microorganisms under study is similar to the effect of usage of known antiseptics such as Lyzoformin 3000 (Germany, Lyzoformin) and Sterillium (Germany, BODE Chemie Hamburg). There is well-known fact that the most of improvers of oxidizing action and preserving agents in bakery production suppress vital activity of yeasts which has a negative impact on biochemical, colloid and

physical-chemical processes during maturation of dough semi-products [8]. One of the reasons of delay in microbiological processes is the presence of AOFs capable of inhibiting the yeast metabolism in such food systems.

**Table 2**

**Effect of plasma-chemically activated water and various antiseptics on growth of microorganisms [7]**

| Microbiological cultures | Delay in microorganisms growth, mm, when using: | | | |
|---|---|---|---|---|
| | plasma-chemically activated water with 500 mg/l concentration of peroxide compounds | hydrogen peroxide solution of 500 mg/l concentration | Sterillium | Lyzoformin 3000 of 1000 mg/l concentration |
| Staphylococcus aureus | 20 | 13 | 13 | 20 |
| Staphylococcus saprophyticus | 10 | N/A | 8 | 13 |
| Staphylococcus epidermidis | 14 | 12 | 11 | 20 |
| Escherichia coli | 13 | 10 | 8 | 15 |
| Pseudomonas aeruginosa | 12 | N/A | 8 | 10 |
| Candida ablicans | 11 | 11 | 13 | 20 |
| Streptococcus pyogenes | 8 | N/A | 20 | 15 |
| Proteus vulgaris | 14 | 8 | 34 | 20 |

Investigation of the effect of water exposed to NCP on sensitivity of bakery yeasts produced by Lviv and Kryvyi Rig yeast plants showed no zones of delay in growth of Saccharomyces cerevisiae culture, that is, the usage of plasma-chemically activated water did not suppress the bakery yeast growth. During experimental investigations it was found that Saccharomyces cerevisiae yeast cultured on Sabouraud broth prepared with the use of plasma-chemically activated water and control samples where the mains drinking water was used for culturing had the same cultural properties, i.e. duration of growth, shape, color, and size of colonies. By way of studying the condition of microorganisms in micro-chambers it was determined that experimental and control samples of bakery yeasts had the same morphology inherent to yeast cells, and the young cells were observed as well.

Effect of specific components of nutrient media on microorganisms depends both on their concentration and duration of action on the objects under study. With the aim of determining the deep and more prolonged effect of water exposed to NCP on bakery yeasts after their culturing on Sabouraud broth for 24 hours with the subsequent three-time passaging the control of microorganisms' condition was carried out. Results of investigations have shown that micro-mycetes cultured in the liquid nutrient media with the

use of plasma-chemically activated water had cultural properties (gas production, turbidity of nutrient media) similar to control samples.

It is impossible to assess the action of impact factors on vital activity of the yeasts without determination of quantity of colony-forming microorganisms. For that purpose, bakery yeasts after their culturing on Sabouraud broth and three-time passaging were seeded on Sabouraud dense nutrient medium and incubated for 24 hours at temperature of 28 °C. Obtained data has shown that in the liquid nutrient media prepared with the use of water exposed to NCP quantity of microorganisms forming the colonies was growing 6,5–15 times compared with the control (Table 3) both for the bakery yeast of TM "Lvivski" and the yeast of TM "Kryvorizki".

**Table 3**

**Effect of plasma-chemically activated water on physiological characteristics of Saccharomyces cerevisiae culture**

| No. of water sample used for yeast culturing | **Bakery yeast of TM** | |
|---|---|---|
| | Lvivski | Kryvorizki |
| 1 | $10^5$ | $10^5$ |
| 2 | $6,5\times10^5$ | $10^6$ |
| 3 | $10^6$ | $10^6$ |
| 4 | $10^6$ | $3\times10^6$ |
| 5 | $10^6$ | $10^6$ |

However, the prevailing effect was observed in case of culturing of the yeast produced by Kryvyi Rig yeast plant in the presence of water exposed to NCP, with the concentration of peroxide compounds of 500 mg/l (sample No. 4). It is evident that such difference is conditioned by peculiarities of fermentative complexes of yeasts typical for a certain strain of microorganisms used under conditions of their industrial production. Cultural properties of Saccharomyces cerevisiae of the above manufacturers for the experimental and control samples featured no difference.

Morphology of Saccharomyces cerevisiae yeast cultured on the liquid nutrient media with introduction of the mains drinking water and water exposed to NCP was studied with the use of phase-contrast microscopy. Fig. 2 shows the morphological state of Saccharomyces cerevisiae cells. Certain peculiar features of the Saccharomyces cerevisiae morphological structure depending on the yeast industrial origin were found: yeast of Lviv yeast plant had more rounded shape (Fig. 2.A, 2.B), while the yeast of Kryvyi Rig yeast plant were of somewhat elongated and oval shape (Fig. 2.C, 2.D). This difference is evidently caused by using of the yeast of various strains under conditions of production at specific company. At the same time, morphology of yeast cells was preserved with the use of water exposed to NCP – both experimental and control samples of microorganisms were characterized by typical appearance and shape (Fig. 2, pos. 1), and young budding cells were also in sight (Fig. 2, pos. 2). Biochemical properties of Saccharomyces cerevisiae yeast were determined on Giss media after their culturing in Sabouraud broth using plasma-chemically activated water and mains water without additional treatment. It is shown that both experimental and control samples fermented glucose, maltose and sucrose and had

similar biochemical properties. No suppression of metabolism of bakery yeast when using water exposed to NCP occurred.

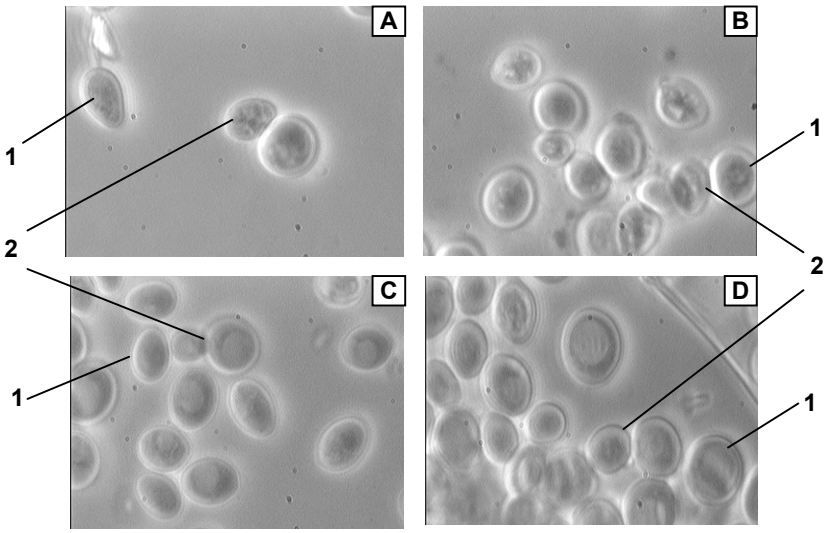


**Fig. 2. Morphology of yeasts of TM "Lvivski" (A, B) and "Kryvorizki" (C, D) with the use of mains water (A, C) and plasma-chemically activated water (B, D): 1 – independent cell; 2 – budding cells.**

Concerning the effect of hydrogen peroxide on the yeast, rather conflicting data is known. Results of investigations of the work [9] prove that with the use of hydrogen peroxide of 100-1000 mg/l concentration fermenting activity of bakery yeast is reduced. However, the paper [10] deals with the method of activation of must fermentation consisting in previous treatment of the yeast with the solution of hydrogen peroxide of 100 mM concentration and addition of the yeast ferment lysate to the initial must. Besides, the effect of parameters of treatment of brewing yeast with hydrogen peroxide which provides for the largest quantity of viable osmo-tolerant cells in suspension was determined. Proposed treatment of the yeast suspension with hydrogen peroxide allowed decreasing the negative impact of higher concentrations of ethyl alcohol on the fermentation process. As it was shown by results of investigations, growth of Saccharomyces cerevisiae produced by Lviv and Kryvyi Rig yeast plants was not restrained because of use of plasma-chemically activated water. On the contrary, increase in quantity of viable yeasts in suspension and stimulatory action of water exposed to NCP on the yeast vital activity was found. It is evident that AOFs, in particular, hydrogen peroxide, have essential influence on the effects obtained. Bailyak [11] has shown that *in vivo* hydrogen peroxide is capable of inactivating or activating antioxidant ferments depending on their concentration, peculiarities of the yeast strain and phase of the culture growth. The ability of baking yeasts to survive in the environment with presence of hydrogen peroxide depends on the capacity of antioxidant systems of cells. In the presence of catalase, with the action of oxidative stress induced by $H_2O_2$, activity of antioxidant systems and cell survival shall increase owing to restoration of intracellular homeostasis. Since water exposed to NCP contains peroxide and superoxide compounds, it should be noted that oxidative processes in Saccharomyces cerevisiae are enhancing, with the further adaptive response of cells. That

is, AOF content in plasma-chemically activated water results in mobilization of physiological and genetic reserves of a cell when the gradual increase in the organism resistance to stress action is displayed as metabolism stimulation and improvement of cultural properties of microorganisms. Besides, usage of plasma-chemically activated water may possibly increase the penetration of water molecules through membranes of Saccharomyces cerevisiae, that in turn speeds up metabolism between the cell and its environment and promotes higher resistance to stress, serving as an impulse to increased reproduction of microorganisms in the nutrient medium.

Results of experimental investigations are important for biotechnology and, in particular, for the theory and practice of bakery production based on microbiological processes traditionally initiated by Saccharomyces cerevisiae yeasts.

## Conclusions

It was determined that plasma-chemically activated water had no suppressive action on Saccharomyces cerevisiae yeast in contrast to its impact on various pathogenic and opportunistic-pathogenic microorganisms.

The experiments prove that with the use of plasma-chemically activated water morphological characteristics and biochemical properties of bakery yeasts produced by Lviv and Kryvyi Rig yeast plants are preserved.

It is found that culturing of Saccharomyces cerevisiae yeast on the nutrient media prepared with the use of water exposed to NCP resulted in 6,5–15 times' increase in quantity of viable microorganisms compared with the control on the mains drinking water, and therefore it is promising for use in the yeast production, baking and fermenting branches of food industry.

Further investigations shall be aimed at determining the effect of plasma-chemically activated water on antioxidant ferments of Saccharomyces cerevisiae and peculiarities of transmembrane transfer of water exposed to NCP through hydrophilic channels of membranes of the bakery yeast.

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