Influence of plant extracts on functional activity and intracellular metabolite of brewing yeast

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[Effects of plant extracts on the yeast]

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Abstract

The aim of research to study the effect of aralia extracts on biological activity and morphology of S. cerevisiae during their life.

Methods and result. The aqueous alcoholic extracts of aralia roots, branches and leaves were used; content of glycosides in the extracts was equal to 0.15-0.12 mg/100 ml, respectively. For determination of the effect of aralia extracts on the biological activity of the yeast we prepared the culture fluids – the sample of the beer wort which was used as a control specimen and three test samples to which aralia extracts were added prior to stationary growth phase. The ability of yeast in the flocculation, number of viable cells, their morphology, intracellular content of glycogen and ultrastructure of yeasts was studied

Result. The cells of test samples had pronounced morphological differences from the cells of control sample which were indicative of intensified metabolic processes and increased period of activity under the influence of aralia extracts. As, the extracts of *Araliaceae* obtained from different organs of the plants are the stimulants of biological activity of *S. cerevisiae* yeast cells, but the action of leaves extracts is more pronounced.

Discussion. This study increases our understanding about influence of biologically active substances plans on the function and activity of yeast. This date can be used in practical applications improve the biotechnological properties of *S. cerevisiae* yeast, race 34/70, contributing to the intensification of the processes of fermentation and improve the quality of finished beveragein the brewing culture, as well as other sectors of the food industry.

Key words: *Saccharomyces cerevisiae*, biologically active substances, plant extracts, aralia

Introduction

Saccharomyces genus, especially *S. cerevisiae* and its relatives, is traditionally associated with the production of alcoholic beverages and bread (Maemure et al, 1998). This yeasts has evolved into an efficient fermentation microorganism that has acquired qualities such as high ethanol productivity, tolerance to process harddiness, tolerance to fermentation by products and is therefore preferred for ethanol production from crops (Zaldivar et al, 2002; Douglas et al, 2006). The brewing process starts with the inoculation of yeast cells in the sweet wort and after its growing, the cell metabolize the sugars into alcohol (called wort attenuation) and impart the desired flavour to the beer. The aim of modern biotechnology in the production of various kinds of drinks is the creation of yeast strains with improved properties. One solution to this problem is to improve the biotechnological properties of S. cerevisiae, contributing to the intensification of the processes of fermentation and improve the quality of finished beverage. At the present time, the difference physicochemical methods for intensification of processes assimilation yeast and increase of their biological activity have been developed (Shreter 2000; Abramov et al. 2009). The most efficient method among them is the use of biologically active substances.

Plants have been the basis of human nutrition for thousands of years and continue to be considered valuable materials in medicines. In natural environment the plants of *Araliaceae* family such as ginseng, aralia and eleuterococcus are widespread and have concentrated resources in the south of the Russian Far East (Zorikov 2008). This plants contain the active ingredients are known as adaptogenes – glycosides; flavonoids, tanning substances, essential oils and polysaccharides (Shreter 2000). These biologically active substances increase the resistance of the human body to the unfavourable environmental conditions, produce mild tonic effect and prolong life. Possibility to use *Araliaceae* as a source of biologically active substances for improving the functional properties of *S. cerevisiae* yeast is a new and timely line of research. In selection of biologically active substances for the yeast we examined separate organs of the Far Eastern wild-growing tree – Japanese angelica tree (*Aralia elata*): leaves, branches and roots. Japanese angelica tree (synonym – Manchurian aralia) is one of the most ancient medicinal and food products. Its roots contain triterpene glycosides which were given the name “aralosides”. Their chemical composition is based on the structure of oleanolic acid. It is known that aralosides are mainly contained in the root cortex. However, these glycosides were also found in other parts and organs of the plant (Izmodenov 2001; Palagina et al, 2009).

The aim of this researcher was to study the effect of plant extracts, contained aralosides, on the morphology and physiological activity of *S. cerevisiae* during the life cycle in beer.

Materials and methods

The industrial-type strains of brewer's yeast *Saccharomycetes cerevisiae*, race 34/70 (Lallemand, Canada), of the first regeneration was used. This yeast is suited for warm fermentation and resistant to alcohol (this is important in selection of biostimulant), can grow with in hard water and quickly ferments the wort. The yeast was qualified as actively propagating microorganisms, contained 50-60% of budding cells, and possessed high fermentative activity and good flocculating power. For analysis under laboratory conditions, liquid cultures were grown with vigorous agitation to early exponential phase in 1% yeast extract, 2% bacto-peptone, 2% glucose.

Yeasts were cultured in a Sabouraud medium at 37 ^{0}C and in a beer medium following the process of started preparation. The yeast concentration was prepared in accordance with the manufacturer's recommendations in the quantity of $1*10^6$ per 5:l of distilled water. This is the quantity was introduced in the concentration of 0.5% from the volume of culture fluid – the beer wort prepared using conventional method.

The technology for obtaining extracts from different organs of *Aralia* (roots, branches, leaves) using various ethanol concentrations in water had already been developed by then (Pomozova, 2002). 60% ethanol was used for obtaining the maximum quantity of extracted substances from aralia roots and branches, 65% ethanol – from aralia leaves.

Quantitative determination of the amount of flavonoids in extracts of *Aralia* conducted in accordance with GOST 21908-93, and the content of these classes of substances is determined by using the HPLC chromatograph for SCL - 10AVP (Shimadzu, Japan). For identification of flavonoids in the raw materials used ready standards are routine and quercetin (Institute of Biochemistry, Novosibirsk). The content of total amount of aralosides in the extracts obtained from roots, leaves and branches was equal to 0.15, 0.09 and 0.12 (mg/100 ml), respectively.

For determination of the effect of aralia extracts on the viability and biological activity of the yeast cells were prepared the following samples. The culture fluid – the sample of the beer wort was used as a control specimen 1, the sample of the beer wort with addition ethanol was used as a control specimen 2. Three test specimens to which were added in equal quantities the ethanol extracts of aralia (1 ml on 1000 ml of beer) after the incipient fermentation (yeast exponential growth phase) and prior to after-fermentation (stationary growth phase) were used. The process of incipient fermentation took for 5 days, after-fermentation lasted – 16 days. The flocculation of cells (yeast death phase) took place in during the next three days.

Viable cells were measured with a microscope after staining with acridine orange hemi(zinc chloride) salt (Sigma catalog No. A6014, U.S.A.). Viability was calculated by dividing the number of viable cells by the total number of cells, with results given as percentages.

To determine the ability of yeast in the flocculation used a method of Helm (Soares 2011), which reveals the time of differences between the flocculent and powdery yeast. The volume of sediment of greater than 5 mm was considered well-flocculated of yeast, if less the cells had weak flocculation ability.

The morphology of yeast cells was studied after staining used a method of Nocht-Maksimov. To identify the volutin of yeast the fixed in the flame smear stained by methylene blue during 3 min. The stain was washed with water and not dried applied to smear a small drop of 1% solution of sulfuric acid. Volutin defined as inclusions of blue-purple color on the little-blue background of the cytoplasm. Granules of carbohydrate (polysaccharide) were detected when cells were treated with Lugol's iodine solution. At the same granules of glycogen in the yeast cells are stained reddish-brown color. The percentage of cells containing granules was measured with a microscope after staining were measured with a microscope after staining

For electronic microscopy preparations, we used a glutaraldehyde-acrolein fixation protocol (Ito and Karnovsky, 1968). This protocol also preserved glycogen well and provided ideal specimens for morphology as well as for cytochemical localiza-

tion of glycogen. Specimens were fixed in 2.25% glutaraldehyde–2% acrolein in 0.1 M sodium cacodylate-HCl buffer (pH 7.4) on ice for 2 h, pelleted in a microfuge (Beckman Instruments, U.S.A.), postfixed for 2 h at 48 ^{0}C in 2% osmium tetroxide in the same buffer, stained in block with Kellenberger's uranyl acetate plus 4% sucrose for 2 h at room temperature, dehydrated in ethanols and propylene oxide, and embedded in Epox 812. The samples were subjected to serial ultrathin sectioning in using ultramicrotome LKB-V (Sweden). Samples were contrasted with lead citrate under the standard method and scanned in a transmission electron microscope JEM-100S (JEOL, Japan) at accelerating voltage equal to 80 kV.

Results

Changes of cell growth during propagation under effect of *Aralia* extracts for control and test specimens of beer are shown in Fig. 1. The differences between the values for control samples 1 and 2 were statistically unreliable. The counting of yeast cells of growth stationary phase showed a definite trend in the control and experimental samples. The maximum of cell growth of all samples reached between 3 days and 6 days, which were similar to the results of Maemura et al. Also, it was determined that in the control sample was a gradual decrease in the number of physiologically active cells from 47% to 15%, which explains the natural moderate decrease of functional activity of the yeast. In the test samples with extracts of *Aralia* initial amount of physiologically active cells was lower than in the controls (average was 33%), which is apparently associated with a certain "shock", obtained by microorganisms from the action of ethanol contained in extracts of *Aralia*. However, in the days of stationary phase, the number of viable cells in all test samples increases (when compared with control) and at the end of the period exceeded the control by an average of 10%. The most pronounced effect on the number of physiologically active microorganisms was observed in the experiment with an extract from the leaves of *Aralia* (Fig. 1b). Investigation of the viability of microorganisms showed that extracts of Aralia significantly inhibit the yeast in the first days after the addition, but do not interfere with normal passage of the technological process.

In studying the process of *S. cerevisiae* flocculation determined that the addition of extracts of aralia extracts after the incipient fermentation (yeast exponential growth phase) and prior to after-fermentation (stationary growth phase) the process of yeast flocculation speeds up. This has a positive effect on the quality of the drink, it becomes more transparent, improve its palatability.

Morphological study showed that *S. cerevisiae* yeast of race 34/70 were round or oval in shape and had the sizes typical for this race. The cells were uniform in size, had thin cell membrane, homogeneous or finely grained cytoplasm and small vacuoles. The examined culture was characterized with high viability and active metabolism. Fig. 2 shows the changes of the intracellular levels of glycogen after propagation of yeasts. The differences between the values for control samples 1 and 2 were statistically unreliable.

As shown in Fig. 2a the quantity of glycogen-containing cells in addition of leaves extracts was significantly higher than in control samples. Also should be noted the displacement in terms of observation and a more stable dynamics of glycogen content in yeast cells. The sample without extracts showed the lowest level of glycogen, whereas the highest level of glycogen

was found for the sample with addition of branches extracts (Fig. 2b), which is explained by the increased growth of yeast cells. All of the yeast strains tested also showed the highest glycogen concentration after propagation and a marked decline after the 1st fermentation, and a little after then. It was also observed after propagation that more glycogen was accumulated in top fermentation yeast. These different results among the quantity of glycogen-containing yeast might be explained by a sensitivity of cells for biologically active substances which contained in extracts. According to Powell et al (2003), the relationship between glycogen dissimilated and sterol formed is stoichiometric. Fermentations of wort pitched with low-glycogen yeast were slower than those pitched with high-glycogen yeast. In the present study, there was direct correlation between the glycogen concentration of pitching yeast and cell growth and flocculation.

As can be seen in Fig. 3a, in the exponential growth phase the control specimen of *S. cerevisiae* showed presence of all organelles typical for the yeast cells. Presence of thin (less than 0.5 mkm thick), compact and elastic cell membrane consisting of cell wall and plasma membrane was indicative on the presence in the culture mostly the young cells (Soares 2011). The cellular cytoplasm was homogeneous and included small-sized vacuoles. The nucleus and nuclear membrane with dense nucleoplasm were clearly seen. The ribosomes had uniform spatial distribution, mitochondria and Golgi body could be seen. The increased quantity of lysosomes and vacuoles which occupied the greater space in the cell from stationary growth phase was observed. By the end of the experiment (on the 24^{th} day) the cells of control sample were in the late stationary phase or phase of death. Local lysis of cytoplasm and numerous huge vacuoles (Fig. 3b) were found in the *S. cerevisiae* cells, enlarged cisterns of endoplasmic reticulum were also noted.

When the extract of *Aralia* was added to the wort, the morphology of *S. cerevisiae* cells was different from that of yeast cells of control sample. For instance, electron-dense areas in the nucleus (Fig. 3c), increased number of ribosomes, presence of microfibrilles, numerous vacuoles and larger area of endoplasmic reticulum were observed in cells. Also, some structures of membrane origin were seen along the inner surface of cytoplasmic membrane. All above-mentioned characteristics suggest the increase in synthetic activity of the cells. In the late stationary phase the yeast cells were mostly oval and round in shape, with singular *S. cerevisiae* cells with curved cell wall (Fig. 3d). It is necessary to note the presence of bud scar indicating the reproductive capability of yeast, while such phenomenon was not observed in the control culture. The cellular cytoplasm was densified and contained microfibrilles and vacuoles of irregular shape and membrane structures. The morphological characteristics of the positive effect of leaves extract on the yeast included the following: homogeneous electronic-dense cytoplasm without various inclusion bodies, straight cell walls and plasma membrane, and unchanged structure of nucleus. All these characteristics pointed at the active functioning of yeast in the exponential growth phase.

Discussion

One of the novel problems of food biotechnology is the development new products, enriched with a variety of biologically active substances. The study and creation of products that contain interconnected with each other nutrients of different nature and structure should be based on reliable information about their physiological effects on the metabolic and

regulatory functions of the body. Given the expanding range of materials for the production of special beers need to pay particular attention to the creation of drinks with a given chemical composition and properties and study of biochemical and microbiological processes during their processing (Mayer and et al, 2001). Among the possible ways to achieve these objectives, as one in greatest significant, it is possible to determine the path of creating a wide range of new special beers containing additives derived from natural plant materials. Beer with such additives, acquires a specific organoleptic and physico-chemical properties, additives affect the nutritional value.

As a supplement, containing biologically active substances, adaptogenic orientation, we used aqueous-alcoholic extracts of the unique wild plants of the Far Eastern Russia. For the preparation of a special beer wort fermentation stage were injected extracts of *Aralia*. Extracts were prepared from roots, leaves and branches of plants. It is shown that to obtain a new special beers efficient use of extracts obtained from renewable raw materials – leaves and branches of *Aralia*.

As can be seen from the above, the examination of *S. cerevisiae* ultrastructure showed that the cells of test specimens had pronounced morphological differences from the cells of control specimen which were indicative of intensification of metabolic processes under the influence of aralia extracts. It should be also noted that introduction of the above extracts resulted in longer period of the yeast's activity.

The positive physiological effect seen in *S. cerevisiae* cells exposed to aralia preparations can be attributed to action of aralosides and flavonoids contained in the extracts. As was shown in the works of I.I. Brekhman (Palagina et al, 2009), the metabolism of biologically active substances contained in *Araliaceae* – glycosides – is manifested in activation of the intracellular hexokinase enzyme system which is the essential and vital component of glycolysis. This results in increased ATP synthesis and ultimately in enhanced general non-specific resistance of cells. The antioxidant properties of flavonoids strengthen this effect (Mingaleeva et al, 2007; Izmodenov 2011). The extracts of *Araliaceae* obtained from different organs of the plants produce the same effect on the yeast cells, but the action of leaves extracts is more pronounced.

In summary, it may be concluded that the plant extracts obtained from aralia are the stimulants of biological activity of *S. cerevisiae* yeast cells.

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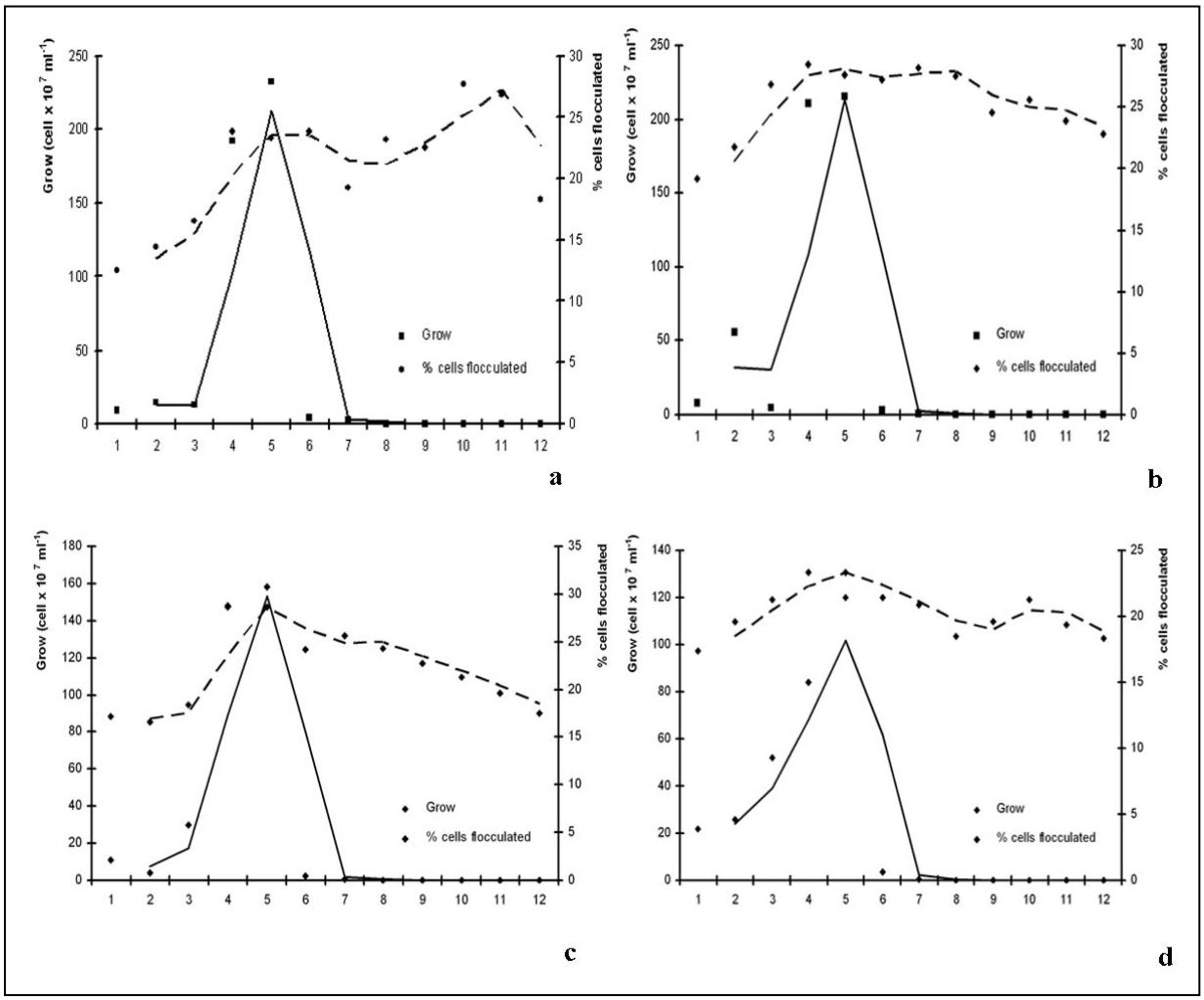

Fig. 1. Changes in the number of viable cells and bottom flocculent yeast: a) control sample without the addition of extract; b) sample with the addition of extract *Aralia* leaves; c) sample with the addition of extract *Aralia* braches; d) sample with the addition of extract *Aralia* roots. The test samples were added in equal quantities the ethanol extracts of aralia (1 ml on 100 ml of beer) after the incipient fermentation (yeast exponential growth phase) and prior to after-fermentation (stationary growth phase).

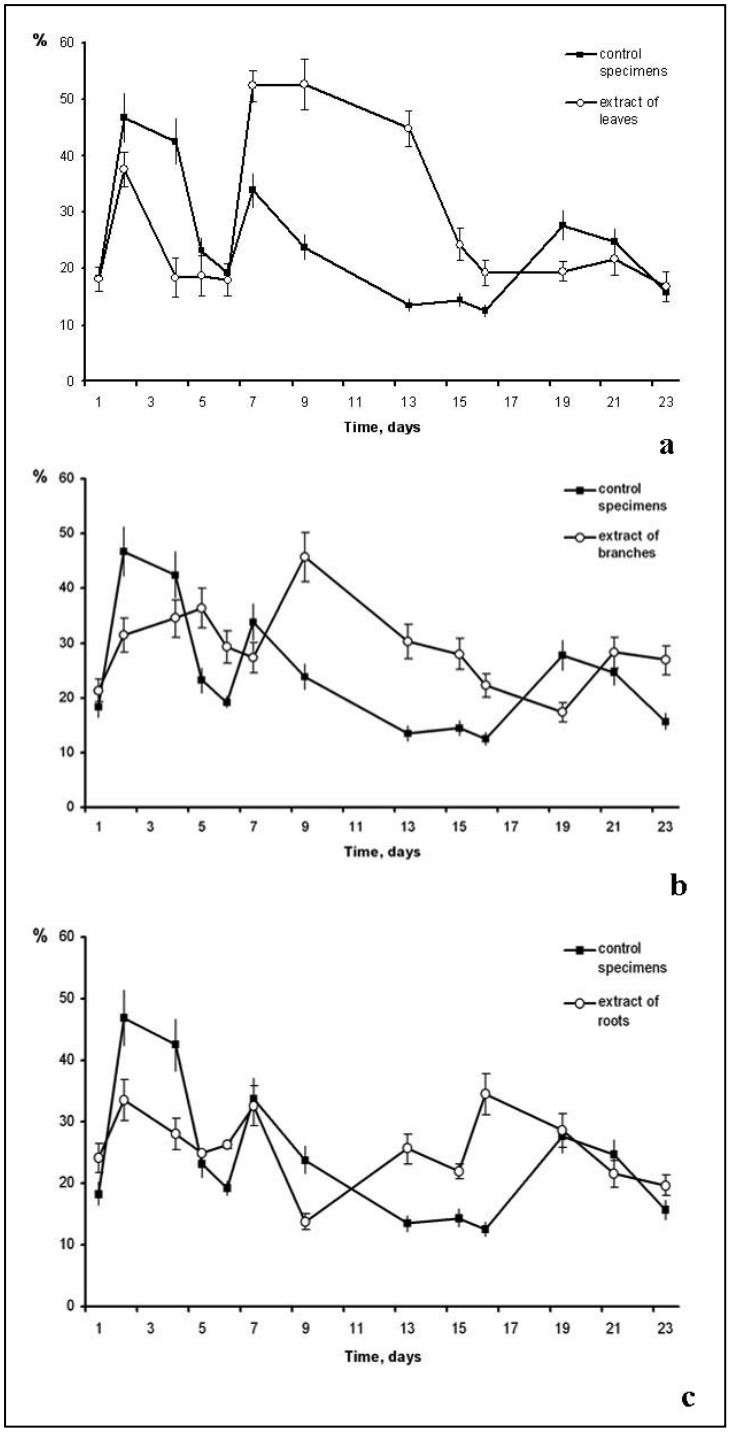

Fig. 2. Changes intracellular levels of glycogen after propagation yeast: a) sample with the addition of extract *Aralia* leaves; b) sample with the addition of extract *Aralia* braches; c) sample with the addition of extract *Aralia* roots. The test samples were added in equal quantities the ethanol extracts of aralia (1 ml on 100 ml of beer) after the incipient fermentation (yeast exponential growth phase) and prior to after-fermentation (stationary growth phase).

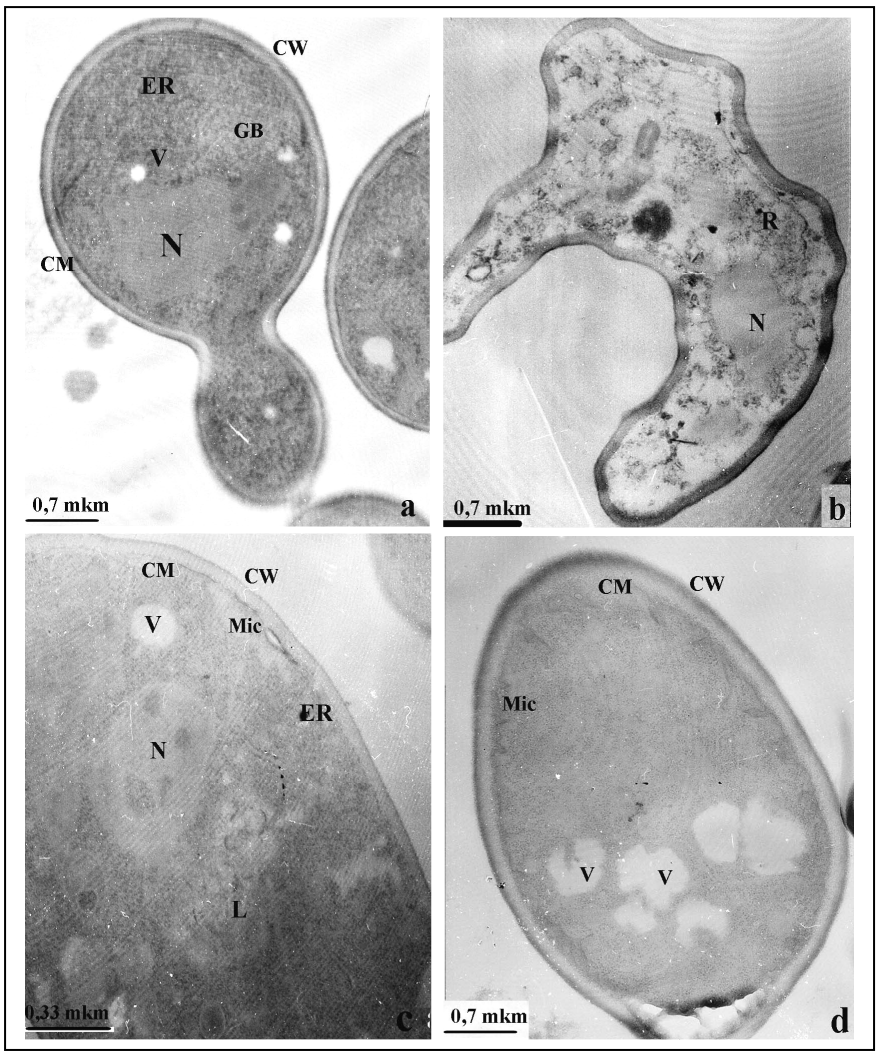

Fig. 3. Transmission Electron Microscopy Images *S. cerevisiae* of sample without addition of extract in the exponential phase and late stationary phase or phase of death (a, b); *S. cerevisiae* in effect of aralia leaves extract from the exponential growth phase and in the late stationary growth phase (c, d).

CM – cell membrane, CW – cell wall, GB – Goldgi body, M – mitochondrion, N – nucleus, ER – endoplasmic reticulum, V – vacuole, R – ribosomes, L – lysosome.