Review article **Open-Access**

FFHD
Functional Foods in Health and Disease

Obtaining yeast mannoproteins with antimicrobial properties

Mariia Kuntsova[1,2], Tatiana Meledina[1], Svetlana Davydenko[2], Dmitrii Manshin[1], Anastasia Andreeva[1]

[1]Faculty of Biotechnologies (BioTech), Saint Petersburg National Research University of Information Technologies, Mechanics and Optics, St. Petersburg, Russia; [2]Innovation and Research Department, Baltika Breweries, Part of the Carlsberg Group, St. Petersburg, Russia

***Corresponding Author:** Mariia Kuntsova, Faculty of Biotechnologies (BioTech), Saint Petersburg National Research University of Information Technologies, Mechanics and Optics, Lomonosova st., 9, 191002 St. Petersburg, Russia

Submission Date: July 28th, 2023; **Acceptance Date:** September 4th, 2023; **Publication Date:** September 15th, 2023

Please cite this article as: Kuntsova M., Meledina T., Davydenko S., Manshin D., Andreeva A. Obtaining yeast mannoproteins with antimicrobial properties. *Functional Foods in Health and Disease* 2023; 13(9): 437-447. DOI: https://www.doi.org/10.31989/ffhd.v13i9.1179

ABSTRACT

Introduction: Mannoproteins (MP) contained in the yeast cell wall (YCW) have high biological activity and possess several techno-functional properties. They are actively used in oenology, as a colloidal stabilizer and an inhibitor of the crystallization of potassium bitartrate, agriculture, as a feed additive for animals, the food industry, as an emulsifier. We obtained MP from *Saccharomyces cerevisiae* yeast and evaluated antimicrobial properties against *Bacillus subtilis* and *Escherichia coli*.

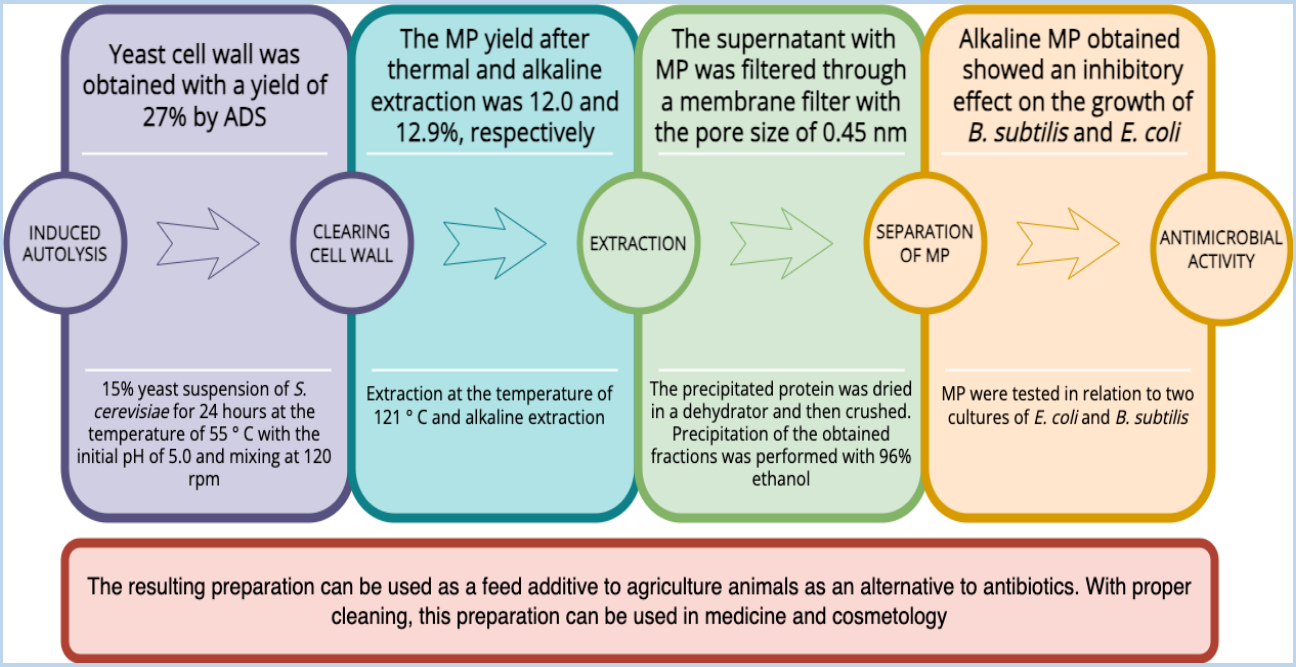
Methods: YCW was obtained by autolysis of 15% yeast suspension of *S. cerevisiae* for 24 h at the temperature of 55° C with the initial pH of 5.0 and mixing at 120 rpm. MP preparations from YCW were obtained in two ways, namely, thermal extraction at the temperature of 121° C and alkaline extraction. The precipitated protein was dried in a dehydrator and then crushed. After that, MP were tested in relation to two cultures of *E. coli* and *B. subtilis* by sowing on liquid and solid nutrient media, with the addition of MP preparation obtained by various methods and different concentrations.

Results: As a result of induced autolysis, YCW was obtained with a yield of 27%. The MP yield after thermal and alkaline extraction was 12.0 and 12.9%, respectively. In relation to *E. coli*, alkaline MP showed the inhibitory ability of 37% at the preparation concentration of 5% (m/v). The fraction obtained by thermal means demonstrated minimal antimicrobial activity (AA). Inhibition was 4% relative to the control sample at the preparation concentration of 3% (m/v). According

to the experimental data obtained with respect to *B. subtilis,* AA of the alkaline (3% m/v) fraction was 80%. The thermal (5% m/v) fraction did not manifest AA, on the contrary, it acted as a catalyst for colony growth.

Conclusions: Mannoprotein obtained by alkaline extraction showed an inhibitory effect on the growth of *B. subtilis* and *E. coli*. The resulting preparation can be used as a feed additive to agriculture animals as an alternative to antibiotics. With proper cleaning, this preparation can be used in medicine and cosmetology.

Keywords: mannoprotein, cell wall, yeast, *Saccharomyces cerevisiae*, antimicrobial properties



INTRODUCTION

Mannoproteins are a part of the yeast cell wall. They are recognized as non-traditional immunostimulants demonstrating antimicrobial, antiviral and antitumor activities [1–3]. Moreover, their antimutagenic and antioxidant properties have been confirmed [4–5] and their effectiveness in wound healing has been proven [6]. Therefore, MP are valuable biologically active raw materials for the pharmaceutical and cosmetology industries [7–9].

Several rheological properties of MP (viscosity, plasticity, elasticity, adhesion) have been well studied and described, which make them a valuable component in the food industry in oenology and in the production of mayonnaise [10–13]. Due to the antimicrobial properties found in MP, these compounds can be used as bioconservants in food products. In animal husbandry, MP are considered substitutes for antibiotics [2]. Agrimos® feed additive containing MP and glucans from *Saccharomyces cerevisiae* is used in artificial aquaculture breeding [14]. The antifungal activity of MP was

established against *Aspergillus flavus* [15]. It should be noted that not only mannoproteins isolated from YCW, but also their complexes with glucan can be in demand in the agricultural, pharmaceutical and food industries [16–17].

Preparations obtained from the same yeast strain, in particular, *S. cerevisiae*, have different antimicrobial effects. For example, the fraction obtained by one type of extraction can positively affect the cultivation of microbial organisms, while the other type affects it negatively. This is probably due to the differences in the chemical composition of MP and the bonds between protein molecules. There are three main types of communication between MP, namely, covalently bound to the structural β - 1,6 - glucan (Pir and GPI), non-covalently bound (captured) to other proteins, and bound to other proteins by disulfide bonds [18]. Each fraction is allocated in a certain way and has individual properties.

There are several ways to extract mannoproteins. The most popular is acid-base extraction [1]. The classical methods are enzyme and heat treatment [19]. Finally, a new method is ultrasonic treatment followed by fermentation [20].

This paper describes in detail the preparation of two MP preparations from YCW *S. cerevisiae* by thermal and alkaline methods. Moreover, antimicrobial activity against *E. coli* has been confirmed and activity against *B. subtilis* has been established. Because it is currently important to isolate MP from yeast to use them in agriculture, food and medicine, their study is timely.

METHODS

Study subjects: Pressed yeast *S. cerevisiae* of *Lesaffre* company was used for the study within the framework of storage life.

Induced autolysis: The first and most important stage of the experiment was autolysis. The subsequent destruction of YCW and the yield of MP depends on how the induced autolysis is performed.

Autolysis is the process of a yeast cell destruction under the action of its own hydrolytic enzymes. Yeast was suspended in distilled water to a dry matter content of 15% in a fermenter (Biostat A, Germany), then we set the following constant parameters: t 55° C, pH 5.0, duration 24 h and stirring 120 rpm. Then, based on these parameters, a graph of the pH dependence on the duration was automatically plotted. After the autolysis time had elapsed, endogenous enzymes were inactivated for 15 min at 80° C. Next, the autolysate was cooled to room temperature *in vivo* and centrifuged (Rotanta 460 Hettich, Germany) to separate the yeast extract from the YCW [21].

The autolysis ratio was defined as the total loss of dried biomass during the process in the following way:

$$R(\%) = \frac{W_0 - W}{W_0} \times 100,$$

where R represents the autolysis ratio, W_0 represents the initial dried biomass, and W represents the residual dried biomass suspension during the induced autolysis [22].

Amine nitrogen in the yeast extract was determined by the method described in [23] and soluble protein by the Lowry method according to the method [24].

Clearing cell wall: An important operation is the washing of YCW from the extract components. To do this, YCW was mixed with distilled water and suspended, after which it was centrifuged for 10 min at 4600 rpm. The procedure was repeated three times until the dry matter content in the washing water is 0.1-0.4%. Then, YCW was

transferred to a sterile container and stored on demand at – 4° C or frozen.

Heat treatment: The next step was thermal extraction. For extraction, it was necessary to prepare a 7.0-phosphate buffer. To do this, 82.4 ml of a solution of 71.5 g/L disodium hydrophosphate dodecahydrate and 17.6 ml of a solution of 21 g/L citric acid monohydrate P were mixed.

YCW was suspended in a phosphate buffer to a dry matter content of 5%, autoclaving (Autoclave Tuttnauer TUT-2540 MK, Israel) is performed at 120° C for 4 h. The resulting suspension was centrifuged at 9800 rpm for 15 min. The supernatant holds MP soluble in hot water, while β-glucans and MP soluble in alkali and other solvents remain in the precipitate [19].

The supernatant was precipitated with 96% ethanol, specifically, it was thoroughly mixed 1:1 with ethyl alcohol, and then maintained at 1.0° C for 24 h. The resulting white sediment at the bottom of the flask was MP. After that, the alcohol was separated, and MP were convectively dried (Case drying ES-4610, Russia) during the day at a temperature no higher than 60° C to prevent the destruction and denaturation of proteins. The mechanism of the alcohol effect can be explained by dehydration of protein micelles, which leads to their reduced stability in solution. Moreover, it is worth considering that with prolonged storage of protein in an organic solvent, the protein denatures and becomes insoluble in the original solvent (alkali / water).

Alkaline extraction: The second part of the YCW was subjected to alkaline extraction in two stages. At the first stage, the YCW were hydrolyzed in a slightly alkaline solution of sodium lauryl sulfate (SDS) to increase the permeability of the wall.

A 5% suspension of YCW in 2% SDS solution was kept in the refrigerator at 4° C for 16 h, after which it was boiled in a water bath with a heating under reflux for 5 min. Next, YCW was separated from the mannoprotein fraction (with disulphide bonds S-S) by centrifugation.

After SDS extraction, the softened walls were subjected to alkaline treatment [25–26]. Extraction was performed in a 3% NaOH solution with a concentration of 37% solid particles. The suspension was boiled with a heating under reflux for 8 h on a magnetic stirrer. At the end of extraction, the suspension was neutralized with sulfuric acid to a neutral litmus reaction. Next, the extract was centrifuged at 4000 rpm for 10 min. The supernatant contains MP covalently bound to structural β - 1.6-glucan and alkali-soluble β - 1.6-glucan; β - 1.3; β - 1.6-glucans insoluble in alkali remain in the sediment.

Finally, the supernatant was precipitated in alcohol as described above and dried.

Determination of antimicrobial activity against *E. coli:* The MP fractions obtained by thermal and alkaline extraction were tested for AA against *E. coli*. *E. coli* was cultured in GMF broth for 24 h at the temperature of 37° C. The number of cells has not been established.

MP with a concentration of 0; 1; 1.5; 2; 5 % were introduced in test tubes with 9 ml of broth. The test tubes were closed with cotton-gauze stoppers, paper caps and sterilized at the temperature of 121° C for 20 min. After the autoclaving incident, the test tubes were cooled to 37° C. In a laminar box under sterile conditions, 1 ml of grown *E. coli* culture was added to each tube, then the samples were thermostated for 24 h at the temperature of 37° C. After cultivation, the spectrum was removed in the wavelength range from 300 to 900 nm. Finally, by changing the optical density index, the increase in biomass was compared.

Determination of antimicrobial activity against *B. subtilis*: *B. subtilis* culture was prepared at the concentration of 10 µl in 1 ml, then diluted in separate Eppendorf tubes 100 and 1000 times. Solutions of MP

fractions, water and SDS solution were pre-sterilized for 20 min at 121° C.

Next, cultivation was performed in test tubes. 910 µl of water / ethanol / SDS / MP solution and 90 µl of *B. subtilis* culture in various concentration were added to each tube, left in the thermostat for 24 h at 28° C. After that, 80 µl of those samples were applied to sterile Petri dishes with YPD and evenly distributed with a Drigalski spatula until they were completely dry and left in a thermostat at 28° C. Three days later, the colonies were counted.

RESULTS AND DISCUSSION

Induced autolysis: In the process of studying the destruction of the cell wall and the production of MP from a YCW, an important question related to the qualitative destruction of cell walls has arisen. In this regard, the time of obtaining YCW with the maximum yield of free amino nitrogen in yeast extract and an average high autolysis coefficient of 24 h was experimentally determined.

The dry matter balance represented in Table 1 was compiled, the equation has the form $a = b + c$, where a is the mass of autolysate, (g); b is the mass of yeast extract, (g); c is the mass of cell walls, (g). On average, the loss of dry matter was 6%.

Table 1. Autolysate dry matter balance

Time, h	Weight of autolysate, g (a)	Mass of yeast extract, g (b)	Mass of cell walls, g (c)	Difference
20	2.0125 ± 0.003	0.5328 ± 0.006	1.3978 ± 0.003	0.0819
24	1.9901 ± 0.008	0.5444 ± 0.002	1.2620 ± 0.005	0.1837

The process of changing the pH of the medium with a change in the duration of induced autolysis is shown in Figure 1. An intriguing observation has emerged in our study: the pH value decreases after 14 hours of autolysis and stabilizes by the 21st hour. This stabilization likely corresponds to the release of acids from the cells. Therefore, it is imperative to assess the presence of volatile organic acids in the yeast extract

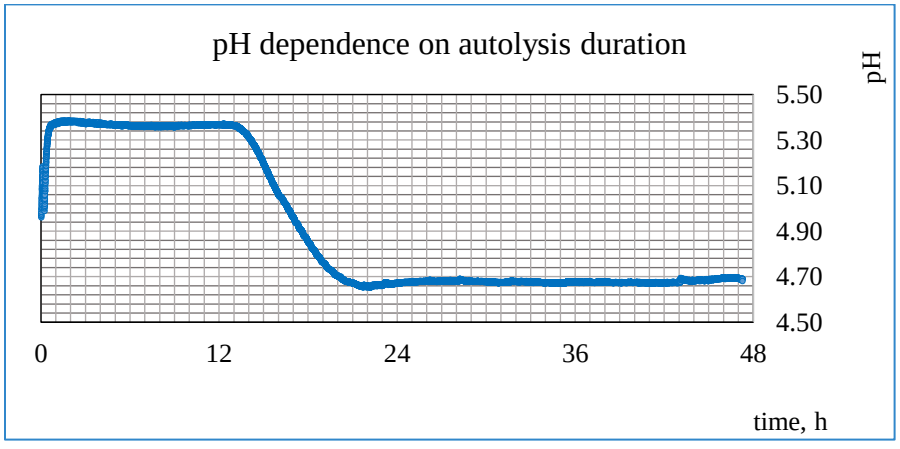


Figure 1. The effect of autolysis duration on the pH of the autolysate

The universal duration of autolysis to obtain MP is 24 h. During this time, all cells are autolyzed, α-amine nitrogen passes into yeast extract. It is not recommended to increase the duration since water-soluble MP can go into the extract. The analysis of the autolysate after 24 h is presented in Table 2.

Table 3 shows the autolysis coefficient at the initial pH of the yeast suspension of 5.0 and 5.5. The destruction of YCW is less effective at a high initial pH. In addition, autolysis proceeds less efficiently in the absence of mixing.

Table 2. Analysis of yeast autolysate

Time, h	R,%	DS yeast extract, brix	pH	FAN yeast extract, 100 mg/L	Lowry's Soluble Protein in yeast extract, mg/ml
0		-	5.00		
12	19.54 ± 0.6	6.2 ± 0.1	5.39 ± 0.1	139.35 ± 4.4	0.012 ± 0.1
16	23.28 ± 0.9	6.3 ± 0.1	5.07 ± 0.2	161.89 ± 3.7	0.014 ± 0.1
20	31.26 ± 1.4	7.0 ± 0.2	4.70 ± 0.2	166.85 ± 2.8	0.016 ± 0.1
24	37.89 ± 1.1	6.9 ± 0.1	4.67 ± 0.1	166.50 ± 3.0	0.018 ± 0.1

Table 3. Experimental data obtained under various conditions of induced autolysis of 15% yeast suspension

Autolysis conditions				R, %
pH	**temperature, ° C**	**time, h**	**rpm**	
5.0	55	24	–	33.6 ± 4.1
5.0	55	48	–	41.2 ± 3.8
5.5	55	24	120	27.5 ± 2.9
5.5	55	48	120	32.6 ± 3.2
5.0	55	24	120	37.9 ± 1.1
5.0	55	48	120	46.8 ± 1.4

Heat treatment

A part of YCW was thermally extracted, protein was precipitated and dried. The resulting water-soluble fraction after precipitation and grinding is demonstrated. in Figure 2. The yield of MP was 12 ± 0.2%. The remaining part of the wall contained β - glucan and MP insoluble in water. The resulting fraction is poorly soluble in water.

Figure 2. Non-covalently bound MP after thermal extraction.

Alkaline extraction: Next, YCW were subjected to SDS extraction, after which they were extracted in NaOH solution. The resulting fraction after drying had a lighter shade than the previous fraction (Figure 3). The yield of MP was 12.8 ± 0.4%. The resulting fraction is very soluble in water.

Figure 3. Alkali–soluble MP covalently bound to the structural β - 1.6 - glucan.

The thermal and alkaline methods of isolation of MP fractions from the cell walls of yeast *S. cerevisiae* have been experimentally tested. The yield of MP was 12 and 12.8% of the ADS YCW, respectively. All fractions did not have a pronounced taste and smell but stuck to the teeth. Establishing the most effective method has yet to be done.

Determination of antimicrobial activity against *E. coli:* These fractions were tested for antimicrobial activity against *E. coli*. Figure 4 demonstrates the results of the antimicrobial potential of MP after alkaline extraction. The graph reveals that with an increase in MP in the GMF broth, the growth of *E. coli* slowed down (the optical density index decreases) by 37%.

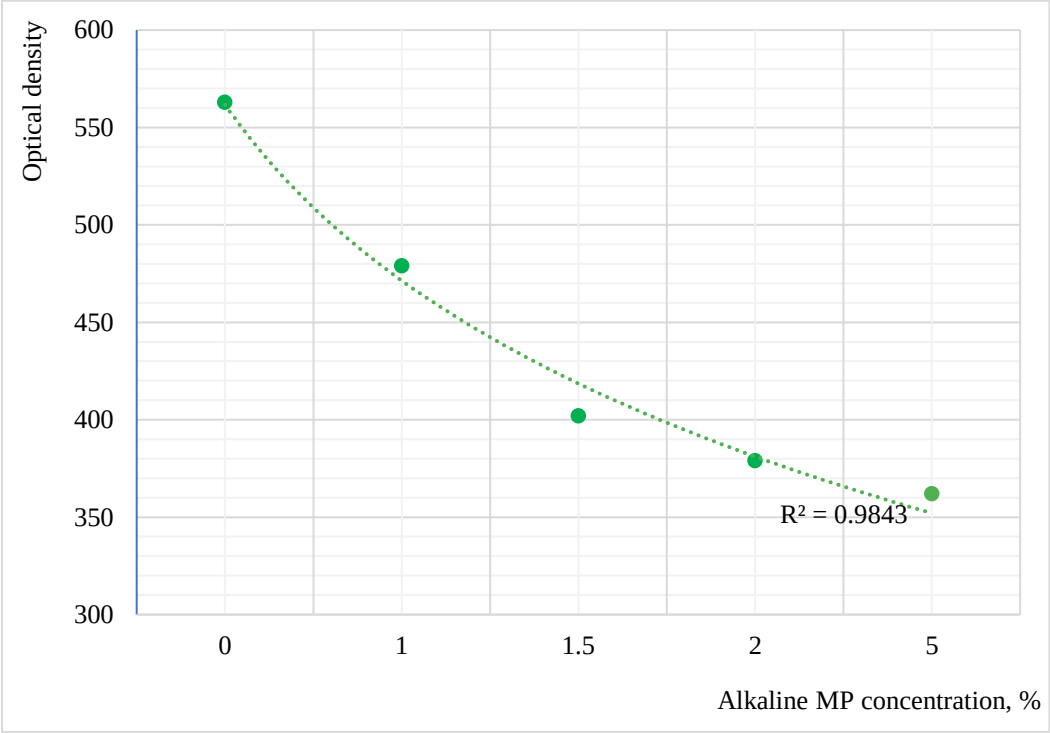


Figure 4. Dependence of the concentration of alkaline MP on the growth of *E. coli* in terms of optical density

On the contrary, during thermal extraction (Figure 5), bacterial growth remained approximately in the same optical density range, which indicates the absence of antimicrobial activity of this fraction.

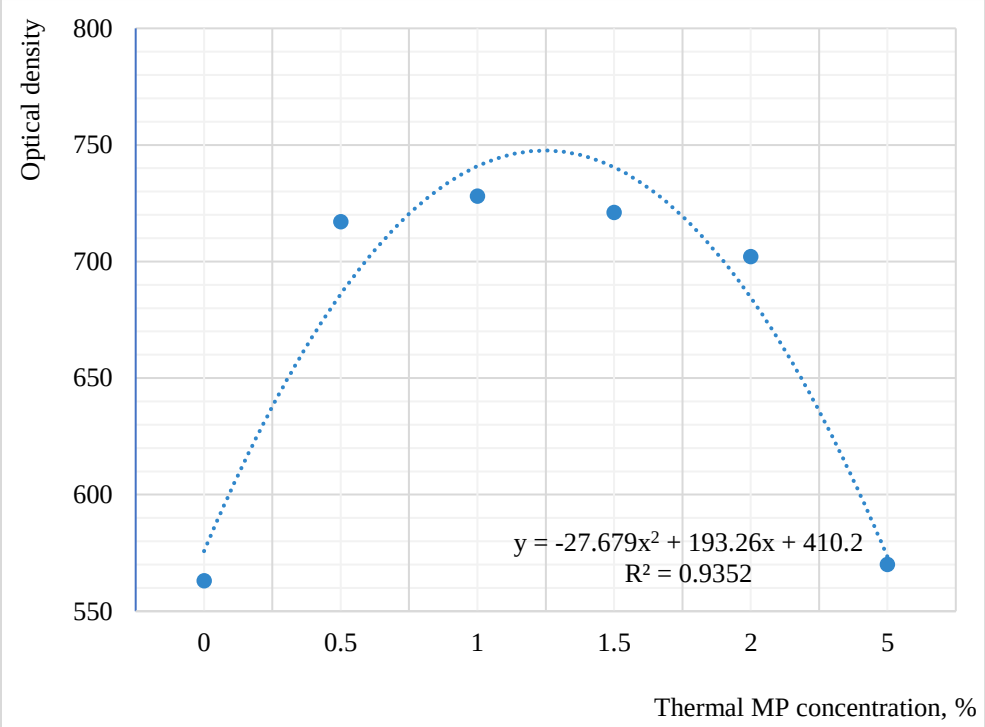


Figure 5. Dependence of the concentration of water-soluble at 121° C MP on the growth of *E. coli* in terms of optical density

Determination of antimicrobial activity against *B. subtilis:* Table 4 shows the counts of colonies on the cups after three-day cultivation. The alkaline preparation MP inhibited the growth of *B. subtilis* by 80%. The thermal preparation, on the contrary, increased the formation of colonies by more than 100%

Table 4. Colonies of *B. subtilis* after three-day incubation

The name of the sample	The concentration of *B. subtilis* is 10 µl in 1 ml	The concentration of *B. subtilis* is 0.1 µl in 1 ml	The concentration of *B. subtilis* is 0.01 µl in 1 ml
9% C_2H_5OH	<120	0	0
H_2O (dist)	448	0	0
Alkali	0	0	0
Thermal MP	>1000	>1000	0
Alkaline MP	90	0	0

The results of sowing after three-day cultivation are shown in Figure 6. The row 1.1-1.3 indicates cultivation with 9% ethyl alcohol; the row 2.1-2.3 – 2% SDS in 3% NaOH (alkali); the row 3.1-3.3 – distilled water; the row 4.1- 4.3 – alkaline MP; the row 5.1-5.3 – thermal MP. The first column (1.1, 2.1, 3.1, 4.1, 5.1) is the concentration of *B. subtilis* 10 µl in 1 ml; the second column (1.2, 2.2, 3.2, 4.2, 5.2) - the concentration of *B. subtilis* 0.1 µl in 1 ml; the third column (1.3, 2.3, 3.3, 4.3, 5.3) - the concentration of *B. subtilis* 0.01 µl in 1 ml. It can be seen from the figure that at the maximum concentration of *B. subtilis* with the addition of alkaline MP, the growth colonies of microorganisms inhibited. On the contrary, thermal MP catalyzes the growth of *B. subtilis*. This is probably due to a change in the structure of the protein when exposed to high temperatures and different molecular weights.

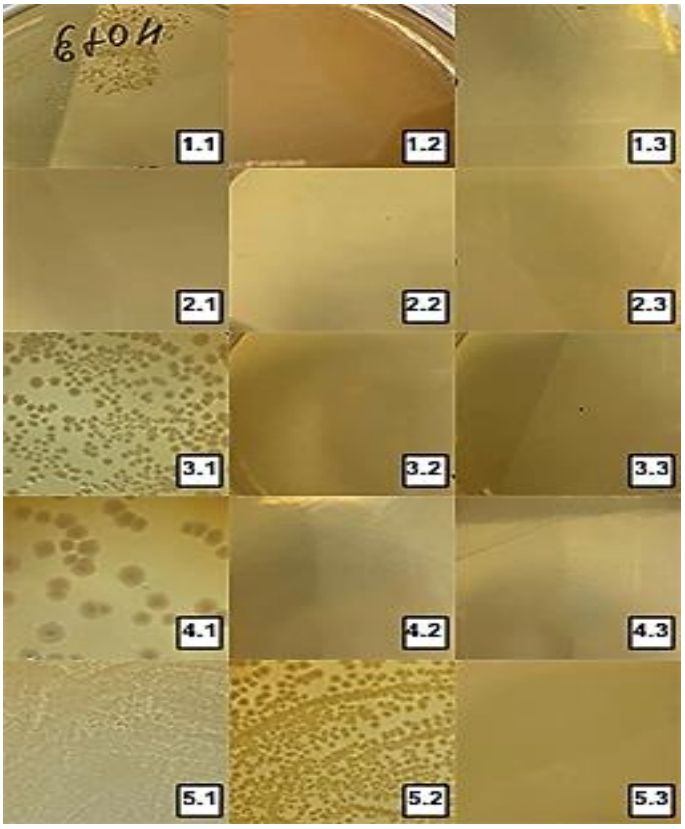


Figure 6. Cultivation of *B. subtilis* under various conditions and different concentrations

AA MP was tested against *E. coli* of two fractions of MP. The alkali-soluble fraction of the preparation inhibits the growth of *E. coli* by 37%. MP obtained by thermal hydrolysis did not affect the cultivation of *E. coli*. With respect to *B. subtilis*, the alkaline preparation inhibited colony growth by 80 %, while the thermal, on the contrary, increased growth by more than 100%. Since yeast MP favorably affects the survival of lactic acid bacteria in artificial gastrointestinal juice and increases their adhesion to Caco-2 cells [27], it has been suggested that the aggregation of lactic acid bacteria (ICD) with yeast in gastric or intestinal juice can increase the tolerance of ICD in the intestine and improve the specificity of adhesion to Caco-2 cells, then the preparation obtained by thermal hydrolysis can be used as a catalyst for the growth of ICD.

The type of extraction influences antimicrobial activity. This is probably due to the molecular weight of the obtained MP. According to other researchers, MP obtained by the enzymatic method also exhibits antimicrobial properties.

CONCLUSION

To sum up, MP obtained by alkaline extraction exhibits an inhibitory effect on the growth of *B. subtilis* and *E. coli*, by 80 and 37%, respectively. The resulting preparation, in addition to the food industry, can be used as a feed additive for farm animals as an alternative to antibiotics, and in aquaculture. With proper cleaning, this preparation can be used in medicine and cosmetology. The preparation obtained by thermal hydrolysis is proposed to be used in the traditional food industry.

List of Abbreviations: MP: mannoprotein, YCW: yeast cell wall, AA: antimicrobial activity, *S. cerevisiae*: *Saccharomyces cerevisiae*, *B. subtilis*: *Bacillus subtilis*, *E.*

coli: *Escherichia coli*, ADS: absolutely dry substances, YPD: yeast extract peptone dextrose, R: autolysis ratio.

Author Contributions: M.K., T.M., S.D. and D.M. designed the research. A.A. provided test yeast and supported the research. M.K. and S.D. conducted the research. M.K. and T.M. wrote the manuscript. A.A. and D.M. translated and formatted the paper. M.K. had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

Competing Interests: There are no conflicts of interest to declare.

Acknowledgements and Funding: We are deeply grateful to *Lesaffre* company for yeast production.

REFERENCES

1. Liu HZ, Liu L, Hui H, Wang Q. Structural characterization and antineoplastic activity of *saccharomyces cerevisiae* mannoprotein. Int J Food Prop 2015; 18: 359–71. DOI: https://doi.org/10.1080/10942912.2013.819364.
2. Bzducha Wrobel A, Farkas P, Chraniuk P, Popielarz D, Synowiec A, Pobiega K, Janowicz M. Antimicrobial and prebiotic activity of mannoproteins isolated from conventional and nonconventional yeast species—the study on selected microorganisms. World J Microbiol Biotechnol 2022; 38: 256. DOI: https://doi.org/10.1007/s11274-022-03448-5.
3. Yehia RS, Saleh AM, Bani Ismail M, Al-Quraishy S, Al-Amri O, Abdel-Gaber R. Isolation and characterization of anti-proliferative and anti-oxidative mannan from *Saccharomyces cerevisiae*. J King Saud Univ Sci 2022; 34: 101774. DOI: https://doi.org/10.1016/j.jksus.2021.101774.
4. Liu Y, Huang G. The derivatization and antioxidant activities of yeast mannan. Int J Biol Macromol 2018; 107: 755–61. DOI: https://doi.org/10.1016/j.ijbiomac.2017.09.055.
5. Chiselita N, Chiselita O, Besliu A, Efremova N, Tofan E, Sprincean A, Danilis M, et al. Biochemical Composition and Antioxidant Activity of Different Preparations from Microbial Waste of the Beer Industry. Acta Universitatis Cibiniensis Series E: Food Technology 2022; 26: 139–46. DOI: https://doi.org/10.2478/aucft-2022-0011.
6. Yoon BH, Lee SM, Chang H-I, Ha CH. Mannoproteins from *Saccharomyces cerevisiae* stimulate angiogenesis by promoting the akt-eNOS signaling pathway in endothelial cells. Biochem Biophys Res Commun 2019; 519: 767–72. DOI: https://doi.org/10.1016/j.bbrc.2019.09.069.
7. Liu Y, Wu Q, Wu X, Algharib SA, Gong F, Hu J, et al. Structure, preparation, modification, and bioactivities of β-glucan and mannan from yeast cell wall: A review. Int J Biol Macromol 2021; 173: 445–56. DOI: https://doi.org/10.1016/j.ijbiomac.2021.01.125.
8. Harbah R, Meledina TV, Manshin DV, Andreev VV. Mannan: structure, biosynthesis, and methods extraction from yeast *Saccharomyces cerevisiae*. Journal International Academy of Refrigeration 2021. DOI: https://doi.org/10.17586/1606-4313-2021-20-1-59-65.
9. Tang N, Wang X, Yang R, Liu Z, Liu Y, Tian J, Luyao X, et al. Extraction, isolation, structural characterization and prebiotic activity of cell wall polysaccharide from *Kluyveromyces marxianus*. Carbohydr Polym 2022; 289: 119457. DOI: https://doi.org/10.1016/j.carbpol.2022.119457.
10. Caridi A. Enological functions of parietal yeast mannoproteins. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology 2006; 89: 417–22. DOI: https://doi.org/10.1007/s10482-005-9050-x.
11. De Iseppi A, Marangon M, Vincenzi S, Lomolino G, Curioni A, Divol B. A novel approach for the valorization of wine lees as a source of compounds able to modify wine properties. LWT 2021; 136: 110274. DOI: https://doi.org/10.1016/j.lwt.2020.110274.
12. Seo S, Karboune S, L'Hocine L, Yaylayan V. Characterization of glycated lysozyme with galactose, galactooligosaccharides and galactan: Effect of glycation on structural and functional properties of conjugates. LWT - Food Science and Technology 2013; 53: 44–53. DOI: https://doi.org/10.1016/j.lwt.2013.02.001.
13. Li J, Karboune S. Characterization of the composition and the techno-functional properties of mannoproteins from Saccharomyces cerevisiae yeast cell walls. Food Chem 2019; 297. DOI: https://doi.org/10.1016/j.foodchem.2019.05.141.
14. Mohamed K, Megahed ME, Ali MAM. Effect of dietary supplementation of Agrimos® on growth performance, feed utilization and immunological parameters of

Macrobrachium *rosenbergii juveniles*. Aquaculture International 2017; 25: 1441–52. DOI: https://doi.org/10.1007/s10499-017-0123-4.

15. Abdolshahi A, Marvdashti LM, Salehi B, Sharifi-Rad M, Ghobakhloo S, Iriti M, Sharifi-Rad J. Antifungal activities of coating incorporated with *Saccharomyces cerevisiae* cell wall mannoprotein on *Aspergillus flavus* growth and aflatoxin production in pistachio (*Pistacia vera* L.). J Food Saf 2019; 39: 12608. DOI: https://doi.org/10.1111/jfs.12608.
16. Wheatcroft R. Production of β-glucan-mannan preparations by autolysis of cells under certain pH, temperature and time conditions. US6444448B1, 2002.
17. Sedmak JJ. Production of beta-glucans and mannans. US8753668B2, 2014.
18. Klis FM, Boorsma A, De Groot PWJ. Cell wall construction in Saccharomyces cerevisiae. Yeast 2006; 23: 185–202. DOI: https://doi.org/10.1002/yea.1349.
19. Li J, Karboune S. A comparative study for the isolation and characterization of mannoproteins from *Saccharomyces cerevisiae* yeast cell wall. Int J Biol Macromol 2018; 119: 654–61. DOI: https://doi.org/10.1016/j.ijbiomac.2018.07.102.
20. Snyman C, Nguela JM, Sieczkowski N, Marangon M, Divol B. Optimised extraction and preliminary characterisation of mannoproteins from non-*saccharomyces* wine yeasts. Foods 2021; 10. DOI: https://doi.org/10.3390/foods10050924.
21. Liu XY, Wang Q, Cui SW, Liu HZ. A new isolation method of β-d-glucans from spent yeast *Saccharomyces cerevisiae*. Food Hydrocoll 2008; 22:239–47. DOI: https://doi.org/10.1016/j.foodhyd.2006.11.008.
22. Wang Y, Yao S, Wu T. Combination of induced autolysis and sodium hypochlorite oxidation for the production of *Saccharomyces cerevisiae* (1-3)-β-D-glucan. World J Microbiol Biotechnol 2003; 19: 947–52. DOI: https://doi.org/10.1023/B:WIBI.0000007328.21451.78.
23. Zdaniewicz M, Satora P, Pater A, Bogacz S. Low Lactic Acid-Producing Strain of *Lachancea thermotolerans* as a New Starter for Beer Production. Biomolecules 2020; 10: 256. DOI: https://doi.org/10.3390/biom10020256.
24. Goldring JPD. Measuring Protein Concentration with Absorbance, Lowry, Bradford Coomassie Blue, or the Smith Bicinchoninic Acid Assay Before Electrophoresis, 2019, p. 31–9. DOI: https://doi.org/10.1007/978-1-4939-8793-1_3.
25. Pitarch A, Nombela C, Gil C. Cell Wall Fractionation for Yeast and Fungal Proteomics, 2008, p. 217–39. DOI: https://doi.org/10.1007/978-1-60327-210-0_19.
26. Kath F, Kulicke W-M. Polymer analytical characterization of glucan and mannan from yeast *Saccharomyces cerevisiae*. Die Angewandte Makromolekulare Chemie 1999; 268: 69–80.
27. Ganan M, Carrascosa AV, De Pascual-Teresa S, Martinez-Rodriguez AJ. Inhibition by Yeast-Derived Mannoproteins of Adherence to and Invasion of Caco-2 Cells by *Campylobacter jejuni*. J Food Prot 2009; 72: 55–9. DOI: https://doi.org/10.4315/0362-028X-72.1.55.