OPTIMIZATION OF THE CONDITIONS REQUIRED FOR CHEMICAL AND BIOLOGICAL MODIFICATION OF THE YEAST WASTE FROM BEER MANUFACTURING TO PRODUCE ADHESIVE COMPOSITIONS

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During the production of beer large amounts of yeast waste are generated. This paper considers the possible making of environmentally friendly adhesive compositions from such wastes. Chemical treatment of yeast wastes increases their adhesive characteristics. Chemical cross-linking with glutaric aldehyde and biological cross-linking by enzyme transglutaminase improves the moisture resistance of the adhesive compositions. In terms of their physical and mechanical parameters they are not inferior to glues of natural origin and can be used for bonding paper, cardboard, and wood. The bonding strength of paper was 421.8 N / m, and that of wood was 27.8 MPa.

Keywords: Yeast; Adhesion; Adhesives; Glutaric aldehyde; Transglutaminase; Glue composition

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INTRODUCTION

Because of stricter environmental requirements for synthetic adhesives environmentally friendly glues and adhesives have in recent years been favored. Production of natural adhesives requires materials of biological origin, such as proteins and polysaccharides. However, due to the complexity of production technology, as well as the nutritional value of the raw materials, the cost of such adhesives is very high. Meanwhile, organic waste of the beer, alcohol, and microbiological industries – such as brewer's yeast, microbial biomass, and distillery grains – generally have very low cost, and they contain significant amounts of proteins and polysaccharides. In Russia, these wastes amount to several hundred thousand pounds per year (Bolshakov et al. 2009; Nenaidenko et al. 2008). At present, existing technologies cannot solve this problem effectively, resulting in the inefficient use of the majority of industrial waste products or their disposal at the expense of the environment. Waste products can be used for the production of non-toxic adhesives (Lambuth 2003; RU patent № 2155790, 2000). Such use has the potential for creation of new products, making many biotechnological processes waste-free. But, in their original form these waste materials are not suitable for the production of adhesives, because most of the chemical groups, which would be involved in the adhesion and interaction with substrates, are stabilized or shielded. To

improve accessibility of these groups and to increase the adhesion of organic waste, it is necessary to optimize the conditions of their modifications. To improve the physicomechanical properties it is necessary to find the optimal component ratio.

The goal of this research was to investigate the possibility of a chemical and biological modification of yeast waste from beer manufacturing to produce useful glue products of various compositions.

MATERIALS AND METHODS

Yeast waste from the beer manufacturing process was the object of the current investigation. The yeast used to manufacture beer was centrifuged at 3000 rpm for 10 min to precipitate cells. The resulting yeast pellets, which had a relative moisture content of 74%, were treated with sodium hydroxide solutions with concentrations of 1, 3, 5, and 7% and with hydrochloric acid solutions with concentrations of 2, 4, 6, and 8% for 15 min and 30 min, respectively. The yeast and corresponding solutions of sodium hydroxide and hydrochloric acid were mixed with a 1:1 mass ratio. After treatment, the yeast solutions were centrifuged at 6000 rpm for 15 min. The resulting chemically modified yeast waste was used to prepare various compositions of glue products. 4% of glycerin weight in weight of modified yeast waste and 0.3% of boric acid weight in weight of modified yeast waste respectively were added as plasticizing and antiseptic agents to prepare the glues. The total quantity and the number of living and whole cells were determined using the cell viability analyzer Vi-CELL and light spectroscopy. The moisture content of the yeast was determined with a thermohydraulic moisture analyzer (AND MS-70) according to the Russian State Standard 14043-80, and the amount of the amino groups was estimated by titration (Rafikov 1978). The viscosity of the glue was estimated using a Rion VT-04R viscometer (Gotech).

The external appearance, color, and odor of the glues were estimated by organoleptic evaluation, according to the Russian State Standard 901-78.

The cohesiveness of the paper was evaluated according to the Russian State Standard 18992-80. For this test, two strips with length of 100 ± 0.5 mm and width of 15 ± 0.5 mm were cut out from paper. One of the strips' area of 20 ± 0.5 mm was covered with a thin layer of glue. Then the second strip was put on evenly. Glued strips were placed under the load and kept for 1 h at room temperature. Before the test, strips should remain at least 12 h without load at 20 ± 2 ºC. Free ends of strips were fixed in the breaking machine and disrupted. The speed of movement of the sliding clamp was 120 mm/min. Adhesive ability P (N/m) is calculated according to the formula,

$$P = F / b \quad (1)$$

where F is the maximum load at which the samples unstuck (N), and b is the width of the strip.

The test was performed with the use of the device Labthink XLW (PC).

The cohesiveness of the wood was evaluated according to the Russian State Standard 3056-90 using a tensile-testing machine (Gotech AI-7000M). The essence of the

method consists of determining the value of the load required to break the adhesive joint that connects two bars of wood. For this test, bars with the width and length of 50 mm and thickness of 25 to 55 mm are used. Solution of glue is applied to the bonding surface of selected pairs of bars evenly. After applying the glue, bars are stacked in pairs and are placed under the press with a uniform distribution of the load of 0.2 MPa (2kgs/cm^2). After 24 h the bars are removed from under the press and left for drying at room temperature for 48 hours. For testing in the wet state, prepared samples are soaked in water at the temperature of 17 ± 2.5ºC while completely immersed in water.

The adhesion of *S* (MPa), is calculated by the formula,

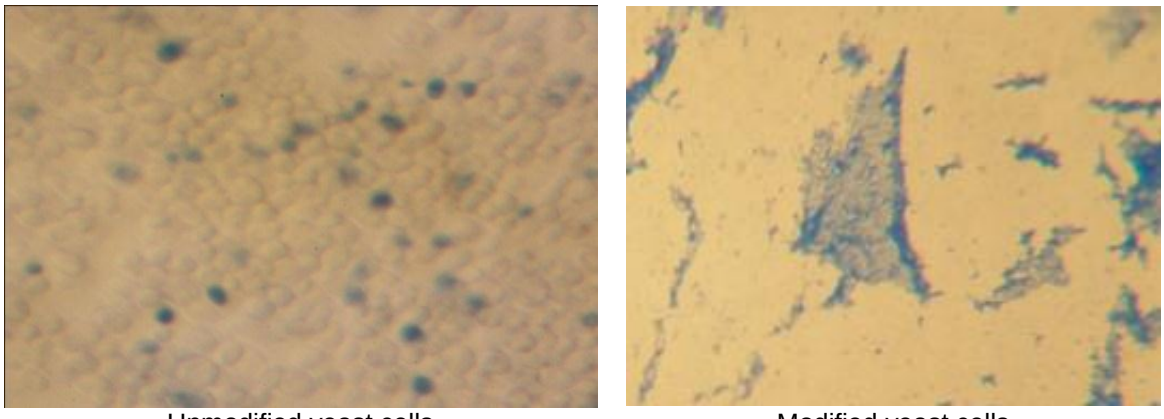
$$S = P / F \quad (2)$$

where *P* is the value of the load at which the sample becomes chipped off (N), and *F* is the area of cleaving (cm^2).

IR spectra were recorded in a thin layer of liquid paraffin using the Fourier transform infrared spectrophotometer IRPrestige-21.

RESULTS AND DISCUSSION

Many traditional glues consist of only one glue substance. Modern composite glues contain different extenders, including plasticizer, filming, waterproofing, and hardening agents that have different chemical structures. The use of these extenders makes it possible to produce adhesive compositions that have the specific properties required for gluing different materials. The yeast waste from the beer industry can be used as substitute for these natural glues. During fermentation of beer, the yeast accumulates inside the fermentation tank. Some of that yeast is used as inoculum, but the rest is waste. This dry-pressed yeast is the by-product of beer manufacturing. Analysis of the chemical composition of the yeast waste indicated the presence of the proteins that have been used traditionally for producing biological adhesives.



Unmodified yeast cells Modified yeast cells

Fig. 1. Effect of the modification of the structure of yeast cells

Figure 1 shows that the precipitated yeast mainly consisted of whole cells and that treatment with water did not disrupt the yeast cells. However, adding hydrochloric acid or sodium hydroxide to the yeast caused lysis of the cells. The extent to which the cells were broken down and/or destroyed depended on the kind of modifier used and its concentration, the yeast-to-modifier ratio, and the time of the treatment with the modifier. Treatment of the yeast with 1% sodium hydroxide and 2% hydrochloric acid for 30 min caused disruption of 50-70% of the yeast cells, and at higher concentrations there was a complete destruction. The resulting modified yeast precipitates were used as biological adhesives.

Yeast precipitates had a viscous consistency and a typical smell associated with the waste from a fermentation process. The color of the precipitates was defined with the used modifier. Treatment of the yeast with sodium hydroxide resulted in the formation of a brown precipitate, whereas treatment with hydrochloric acid resulted in the formation of the light-brown precipitate. State standards require that glues with different viscosities be used for gluing different materials. For example, the use of glue with a viscosity of 40 to 50 dPa·s is recommended for gluing paper. Gluing wood requires the use of glue that has a viscosity of 90 to 100 dPa·s (Rowell 2005). Also, the glues must have a certain plasticity and resistance to decay (State Standard 3252-80). In the current work, the preparation of glue compositions on the basis of the received precipitates was conducted with glycerin as the plasticizer (Thawien Bourtom 2008) and boric acid as the antiseptic agent. Adding water regulated the viscosities of the resulting glue compositions.

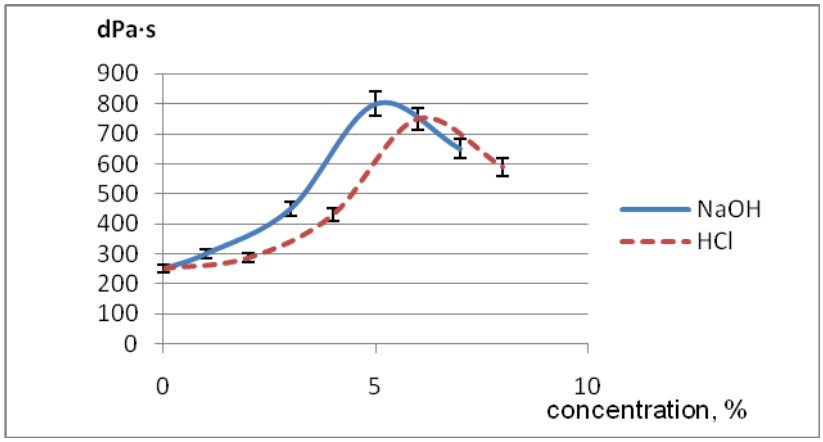


Fig. 2. Effect of the modified concentrations on glue viscosity

The resulting glue samples were characterized by their increased biostability to microbial contamination over a 30-day period. During storage of the glues, no putrid smell developed, the viscosities did not change, and exfoliation did not occur. These observations indicate that the resulting glue compositions could be stored for a long period of time without losing their commercial viability. The research results related to the strength of the paper gluing showed that the adhesive properties of the beer yeast varied depending on whether hydrochloric acid or sodium hydroxide was used in the preparation. The effects of the chemical modifiers in varied concentrations are shown in Table 1.

Table 1. Tensile Strength of the Adhesive Compositions as Determined by Separating the Glued Papers (State Standard 18 992–80)

Composition of the glue	Breakdown force (N/m)
Control – beer yeast pellets without treatment	104±54
Glue composition for the beer yeast treated with 1% NaOH	198±10
Glue composition for the beer yeast treated with 3% NaOH	296±15
Glue composition for the beer yeast treated with 5% NaOH	422±21
Glue composition for the beer yeast treated with 7% NaOH	139±7
Glue composition for the beer yeast treated with 2% HCl	178±9
Glue composition for the beer yeast treated with 4% HCl	283±14
Glue composition for the beer yeast treated with 6% HCl	406±20
Glue composition for the beer yeast treated with 8% HCl	159±8

According to the State Standard 901-78, the glue used to glue paper must have a strength of at least 400 N/m. Table 1 shows that modifying the residual beer yeast pellets with NaOH and HCl resulted in a three-fold increase in the adhesive properties of the glue. Glue compositions produced by treating the yeast pellets with 5% sodium hydroxide and 6% hydrochloric acid had the best adhesive properties (Table 1). The higher adhesive properties of the beer yeast after treatment with NaOH and HCl can be explained by the increased availability of the functional groups of the proteins after destruction of the cell walls of the yeast. As we suggested above, further increases of the alkaline and acid concentrations resulted in significant decreases in the adhesive capacities of the glues. Perhaps this effect can be explained by the alterations of the protein conformations that were in stable conditions after treatment with the low concentrations of NaOH and HCl. Further increasing of the NaOH and HCl concentrations causes partial hydrolysis and disturbance of the protein structure. The data in Table 1 agree with this suggested hypothesis. Using higher concentrations of the modifiers resulted in decreasing adhesive capacity of yeast pellets, so the products were less viscous and had a less solid consistency.

It is known that natural adhesives have some advantages with respect to ecological safety compared to synthetic adhesives. However, almost all natural adhesives have one significant disadvantage, i.e., they absorb water. Various additives can be used to increase their water resistances, but such additives are not always effective, and they increase the cost of the adhesives. It is known that glutaric aldehyde is used to create crosslinking, which increases the strength and stability due to the immobilization of the enzyme. Glutaric aldehyde has two functional groups that can react with amino groups to form azomethine bonds (Migneault et al. 2004). Transglutominase is an enzyme that catalyzes the formation of the bond between the carboxyl groups of glutamine and the amino groups of lysine (Gembeh et al. 2005). This enzyme can be used to increase the density and solidity of some foods.

Different volumes of 5% glutaric aldehyde were added to the glues produced by the modification of the yeast pellets with NaOH because azomethine bonds in the acid

condition are not strong. Adding glutaric aldehyde changed the color of the glue to a reddish one.

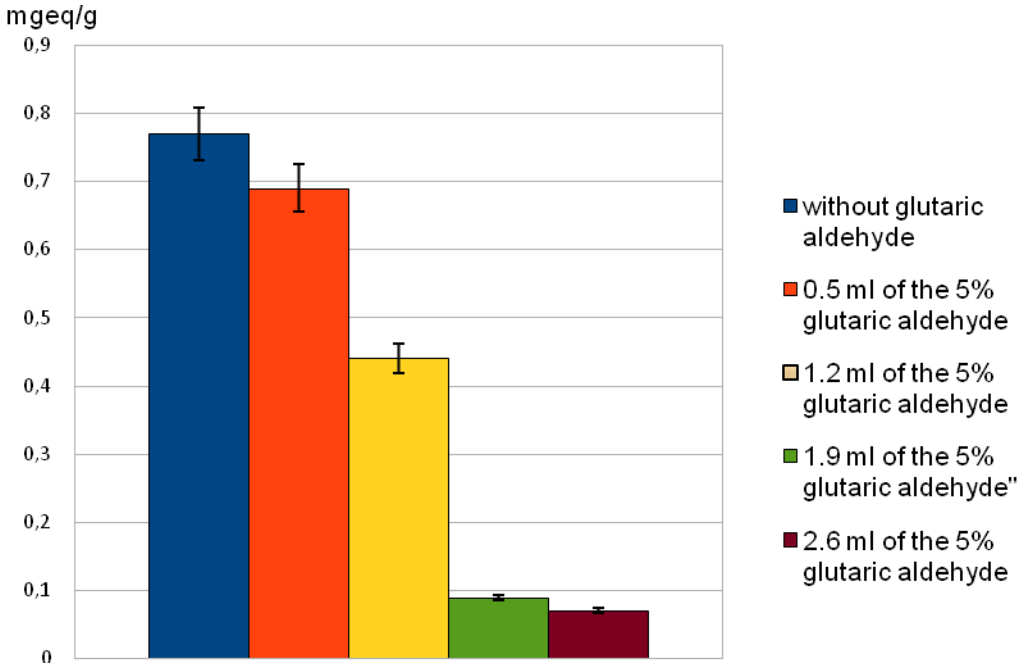


Fig. 3. Changes in the number of amino groups after treatment of the glues with glutaric aldehyde

Experiments in which glutaric aldehyde was added to the modified yeast pellets resulted in decreasing the content of the available amino groups as compared with a control experiment in which no cross-linking agent was used (Fig. 3). The quantity of the available amino groups on the modified yeast pellets before treatment with glutaric aldehyde was 0.77 µgeq/g of the mixture. After addition of 1.2 mL of glutaric aldehyde, the content of the available amino groups decreased by 43% to 0.44 µgeq/g. Increasing the amount of glutaric aldehyde added to the modified yeast pellets to 1.9 and 2.6 mL resulted in the decrease of the available amino groups to the minimal amount (Fig. 3). The IR spectra show that the presence of glutaric aldehyde reduces the intensity of the absorption bands at frequencies 3500 cm^{-1} and 1640 cm^{-1} caused by free amino groups. (Fig. 4). Glutaric aldehyde crosslinks protein to amino groups, thereby reducing the degree of hydration and water solubility of adhesive compositions. Both the treated and untreated (with glutaric aldehyde) modified yeast pellets were spherical in shape, and they were placed under water for 24 h to investigate the effect of the glutaric aldehyde on the water resistance of the resulting modified yeast pellets (Fig. 5). Changes in the structure and shape were observed visually. The samples that had not been treated with glutaric aldehyde collapsed in 2 h, but the samples treated with glutaric aldehyde maintained their structures for 10 to 24 h, depending on the amount of glutaric aldehyde that was added.

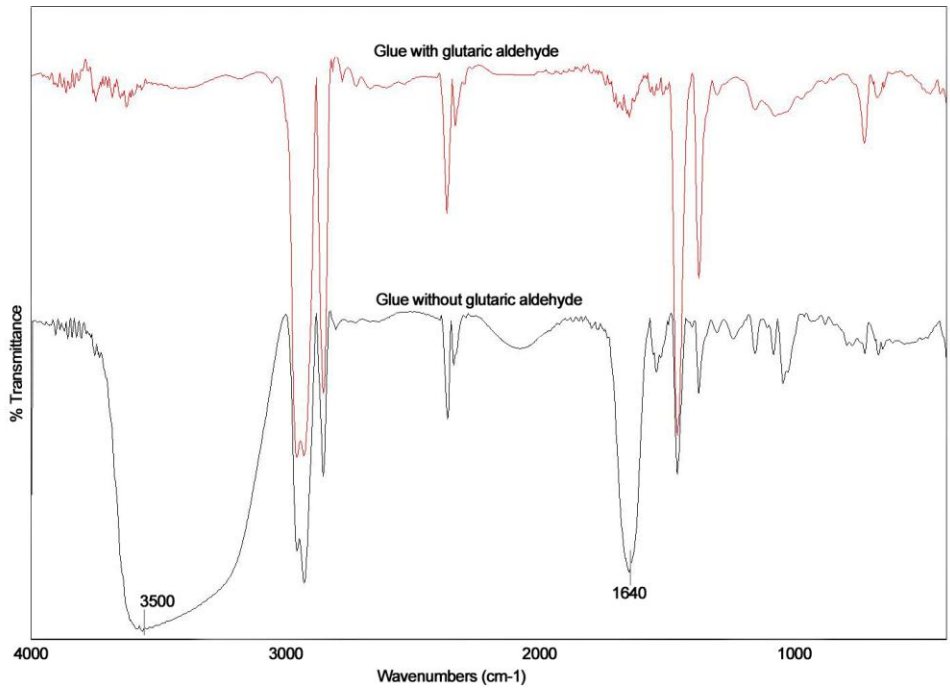
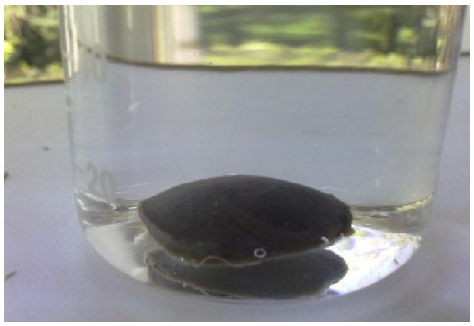
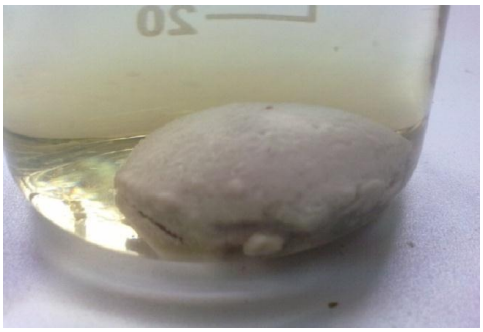


Fig. 4. IR spectra of the glues with and without glutaric aldehyde



Glue to which 1.2 mL of 5% glutaric aldehyde were added just after it was placed in water



Glue to which 1.2 mL of 5% glutaric aldehyde were added after 24 h of soaking in water

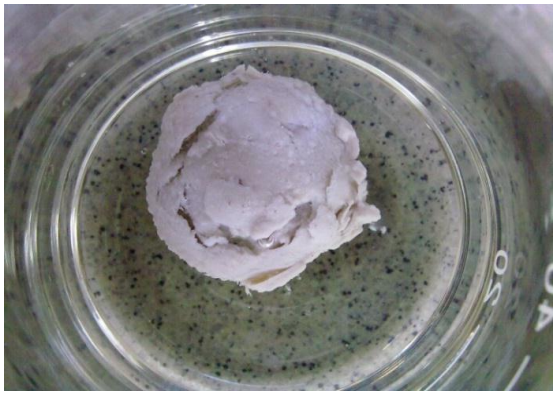
Glue to which no glutaric aldehyde was added just after it was placed in the water

Glue to which no glutaric aldehyde was added after 24 h of soaking in water

Fig. 5. Water resistance of glue treated with glutaric aldehyde as determined by soaking in water

Transglutaminase (enzyme Probind TX, BDF Natural Ingredients, Spain) was added in the amount of 0.3 g per 100 g of the glue (Mirzaei 2011). The efficiency of the transglutaminase was estimated by the amount of ammonia that escaped and reacted with Nessler's reagent (Handbook of AtmosphericPollution Control, 1991; Cardamone 2007). Samples treated with transglutaminase were more resistant to water than control samples, but the total effect was not significant. Increasing water resistance required the addition of significant quantities of the enzyme.

Sample treated with transglutaminase just after it was placed in water



Sample treated with transglutaminase after soaking in water for 24 h

Control sample without transglutaminase treatment just after it was placed in water

Control sample without transglutaminase treatment after soaking in water for 24 h

Fig. 6. Effect of transglutaminase on the waterresistance of glue

The water resistance tests were also conducted for wood. The properties of wood differ from the properties of paper, and, therefore, the adhesive properties of the glue can be different when these two materials are glued together. Gluing the wood allowed us to check the water resistance of the cemented joint, which was conducted after standard drying of the glued samples and after soaking in water for 2 h. Table 2 shows the results of the gluing of the wood.

The data presented in Table 2 indicate that all samples of wood materials had high adhesive strengths that met the requirements of the State Standard for adhesive strength. After soaking in water for 2 h, the samples prepared without adding glutaric aldehyde collapsed under low external pressure. Adhesive strength was increased to 19.9 MPa

before soaking in water and to 10.0 MPa after soaking in water after addition of 0.5 mL of glutaric aldehyde to 5 g of the glue. Addition of 1.2 mL of glutaric aldehyde to 5 g of the glue allowed attainment of the maximal adhesive strength, i.e., 27.8 MPa before soaking in water and 17.0 MPa after soaking in water for 2 h.

Table 2. Determination of Bond Strength of Modified Adhesive Compositions in Accordance with State Standard 2067-93

Composition of the glue	Breakdown force, MPa		
	Beginning of soaking in water	After 1 h of soaking in water	After 2 h of soaking in water
Received yeast pellet	3.6±0.18	-	-
Modified yeast cells without glutaric aldehyde	11.7±1.4	-	-
Modified yeast cells with 0.5 mL of 5% glutaric aldehyde added	19.9±1.5	16.5±0.8	10.0±1.2
Modified yeast cells with 1.2 mL of 5% glutaric aldehyde added	27.8±1.2	23.8±1.2	17.0±1.8
Modified yeast cells with 1.9 mL of 5% glutaric aldehyde added	9.2±0.5	-	-
Modified yeast cells with 2.6 mL of 5% glutaric aldehyde added	7.5±0.4	-	-
Modified yeast cells treated with transglutaminase	20.4±1.1	15.7±0.8	8.5±0.4

The data presented in Table 2 indicate that all the wood samples had high adhesive strengths that met or exceeded the requirements of the State Standard for adhesive strength. After 2 h of soaking in water, the samples prepared without adding glutaric aldehyde collapsed under low external pressure. After addition of 0.5 mL of glutaric aldehyde to 5 g of the glue, adhesive strength was increased to 19.9 MPa before soaking in water and to 10.0 MPa after soaking in water. The addition of 1.2 mL of glutaric aldehyde to 5 g of the glue resulted in the maximum adhesive strength, i.e., 27.8 MPa before soaking in water and 17.0 MPa after soaking in water for 2 h.

Further increases in the quantity of glutaric aldehyde added decreased the adhesive strength of the cemented joint both before and after soaking in water. It is probable that the presence of the excess cross-linking agent blocked all functional amino groups, which are participants in the adhesive process.

CONCLUSIONS

Thus, the chemical pretreatment of yeast waste with the addition of plasticizer and antiseptic can produce glue compositions for different purposes. Chemical and biological cross-linking increases the water resistance of the adhesive. This technology will encourage the use of bioresources, reduce the level of industrial pollution of the

environment, and improve the profitability of enterprises by manufacturing new products from waste.

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