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Characterization and application of spent brewer's yeast for silver nanoparticles synthesis

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The brewer's yeast is an abundant and valuable by-product of the beer industry. In the last years numerous studies have focused on different possibilities for its valorization. The aim of the present study was to characterize spent brewer's yeast and to investigate its application as a reducing agent with combination of extracts of *Rosa damascena* wastes for synthesis of silver nanoparticles (AgNPs). The non-pasteurized and pasteurized brewer's yeast was found to contain small amounts of phenolic compounds: 8.40 and 1.53 μmolGAE/100 mL, respectively, but they were rich in carbohydrates (40.20±1.5%), proteins (41.33±0.11%) and dietary fibers (23.94±1.5%). The water extract of *Rosa damascena* waste was able to reduce the Ag+ quickly and AgNPs with sphere-like shape were formed. This is typical for the AgNPs obtained by reduction with extracts rich in polyphenols. The pasteurized and non-pasteurized brewer's yeasts induced the AgNPs synthesis (proved by visual observation and by UV-Vis measurements) relatively slowly and the average particle size was smaller than that of the particles synthesized by water extract of *Rosa damascena* waste. The combinations of pasteurized and non-pasteurized brewer's yeast with water extract of *Rosa damascena* waste were able to produce AgNPs relatively quickly. The transmission electron microscope images confirmed the observed synthesis of AgNPs.

Keywords: brewer's yeast, *Rosa damascena*, silver nanoparticles, "green" synthesis, waste valorization, polyphenols.

INTRODUCTION

Valorization of biodegradable food wastes became an important issue in the last years. The spent brewer's yeast is a valuable biomass and represents 1.7-2.3 g/L beer. The major species used for bottom fermenting lager beers are *Saccharomyces pastorianus* or *Saccharomyces carlsbergensis* strains. The brewer's yeast was found to be rich in glucans and manoproteins with potential biological activity [1]. Due to the higher amounts of proteins and dietary fibers brewer's yeast is suitable for functional foods formulations [2], and was also investigated as additive for fermentation and production of bread [3]. With its various applications in the food, agricultural and industrial sector the brewer's biomass was proven as a valuable renewable material [4, 5].

In the last two decades nanomaterials and nanoparticles (NPs) with their unique properties (high surface to volume ratio, enhanced surface reactivity or increased ion release, etc.) became a base for obtaining of new improved biosensors, catalyzers, antimicrobial agents, etc. [6]. An alternative and novel approach for obtaining of NPs is the so-called "green" synthesis based on the reduction of metal cations with natural reducing agents, such as: plant extracts, fungi, microorganisms, waste materials, etc. The advantage of this method consists in the single stage environmentally procedure with renewable and usually abundant, cheap materials. Various raw materials and wastes were investigated but experiments with combined wastes and microorganisms are scarce. Beside, this approach allows more complete utilization and valorization of the wastes. This observation gave ground to the purpose of the present study: to investigate and characterize spent brewer's yeast, to utilize it along with fresh brewer's yeast and with combination of *Rosa damascena* waste for synthesis of silver nanoparticles.

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Table 1. Combinations of 0.01M $AgNO_3$, water, *Rosa damascena* extract and brewer's yeasts for different samples investigated.

Sample	*Rosa damascena* extract, mL	Water, mL	Pasteurized brewer's yeast, mL	Non-pasteurized brewer's yeast, mL	0.01M $AgNO_3$, mL
0B	0.1	0.1	-	-	0.3
1B	-	0.1	0.1	-	0.3
2B	-	0.1	-	0.1	0.3
3B	0.1	0.1	0.1	-	0.3
4B	0.1	0.1	-	0.1	0.3

EXPERIMENTAL

Waste materials

The *Rosa damascena* wastes were obtained from Mirkovo distillery (Mirkovo, region of Sofia, Bulgaria, 2017 harvest). The wastes were inspected for impurities, dried at 50°C, and kept at -18°C until further treatment. The brewer's yeasts (both pasteurized and non-pasteurized) were obtained from ABM Production (Plovdiv, Bulgaria). Prior to synthesis the pasteurized brewer's yeast was dissolved overnight in distilled water at 1% w/v and then filtered. The non-pasteurized brewer's yeast was diluted with distilled water (1:13) to 1% dry matter.

Extraction of Rosa Damascena waste with distilled water

150 g dry residues were treated with 1000 mL of water for 1 h at 60°C then left for 24 h at room temperature at constant stirring. The mass was filtered and the insoluble residue was extracted with additional 500 mL of water at the same conditions.

The "green" synthesis of AgNPs was performed by mixing the necessary amounts of 0.01M $AgNO_3$ (Merck, Germany), water, *Rosa damascena* extract and brewer's yeasts according to Table 1.

Analytical methods

The total polyphenol content of ethanolic extracts was determined as described by Singleton and Rossi [7]. The antioxidant activity by DPPH and FRAP assays was determined as described by Slavov *et al*. [8]. The protein content in the brewer's yeast was determined by the Kjeldahl method with automated nitrogen analyzer UDK152 (Velp Scientifica, Italia) using a correction factor N×6.25 for calculation of total protein. Crude lipid content was determined according to AOAC 922.06 method. The total dietary fibers (TDF) were determined by the enzymatic-gravimetric method, using the total dietary fiber assay kit Bioquant 1.12979.0001 (Merck, Germany) according to manufacturer's instructions. The total ash content was determined by igniting 5 g of sample in a muffle furnace at 605°C until constant weight. The total carbohydrate quantity of wastes was calculated by difference after determination of protein, crude lipid, lignin, total polyphenols, and ash quantities. The moisture content was determined with Kern MLB 50-3 moisture analyzer (Kern & Sohn GmbH, Germany). The individual amino acids were determined after derivatization [9] using the HPLC system ELITE LaChrome (Hitachi) equipped with diode array detector Elite LaChrome L-2455. The separation was performed on AccQ-TagTM (3.9×150 mm) column. The contents of calcium, magnesium, iron, copper and manganese were determined according to AOAC 2014.004.

UV-Vis measurements of the AgNPs synthesis were carried out on a Helios Omega spectrophotometer (Thermo Scientific, Madison, WI, USA) equipped with VISIONlite software and operated at a resolution of 1 nm. The scanning was performed in the 350-700 nm range at a scan speed of 1600 nm min^{-1}. The samples of the AgNPs were prepared for TEM analysis by placing a drop of the suspension on a standard copper grid, covered by amorphous carbon layer and allowing the water to evaporate at room temperature. Bright field TEM and Selected Area Electron Diffraction (SAED) images were acquired with the high-resolution transmission electron microscope JEOL JEM 2100 (JEOL, Japan) at an accelerating voltage of 200 kV. Statistical analysis of AgNPs particles size distribution was carried out with Image J software. The identification of the phase composition of the samples was achieved using PDF-2 Database of the International Center for Diffraction Data (ICDD).

Statistical analysis

All analyses were performed in triplicate and the results were presented as mean values. Statistical differences were detected by analysis of variance (ANOVA, Tukey's test) and a value of $p<0.05$ indicated statistical significance.

RESULTS AND DISCUSSION

The brewer's yeasts were investigated for their total polyphenol content and antioxidant activity. Having in mind that the polyphenols are among the major substances responsible for green synthesis of metal NPs, this information is related to the

capability for biogenic synthesis. The results from the analysis are shown in Table 2.

The results suggested that both brewer's yeasts are poor in polyphenolic substances and this also reflects on the observed antioxidant activities. The water extract of *Rosa damascena* waste was rich in polyphenolic compounds: 133.70±0.11 μmol GAE/100 g waste and it confirmed the observation made by Slavov *et al*. [8] that the higher the concentration of polyphenols, the higher is the antioxidant activity: the DPPH method showed 8550±0.98 μmol TE/100 g waste and FRAP – 905.83±0.99 μmol TE/100 g waste.

Furthermore we investigated the overall composition of the brewer's yeasts and the results are presented in Table 3.

Both brewer's yeasts were rich in carbohydrates and proteins while the crude lipids were relatively low: less than 1 %. On contrary, Blagović *et al*. [10] found around 4% in the *Saccharomyces uvarum* yeast obtained as a by-product of industrial beer production. Calcium was the predominating metal and the amounts of dietary fibers were between 21-24 %.

Table 2. Polyphenols and antioxidant activity of brewer's yeasts

Brewer's yeast	Total phenolics, μmol GAE/100 g	FRAP, μmol TE/100 g	DPPH, μmol TE/100 g
Pasteurized	1.53±0.09 [a]	2.45±0.11 [a]	2.05±0.13 [a]
Non-pasteurized	8.40±0.12 [b]	13.40±0.45 [b]	12.00±0.21 [b]

[a, b] – different letters in a row indicate statistically different values ($p<0.05$; ANOVA, Tukey's test).

Table 3. Proximate composition of the brewer's yeasts

Substances	Brewer's yeast	
	Pasteurized	Non-pasteurized
Total carbohydrates, % DM	40.20 ± 1.5 [a]	38.61±1.39 [a]
Moisture, %	2.00 ± 0.7	-
Crude lipids	0.08 ± 0.03 [a]	0.09 ± 0.02 [a]
Ash content, %	0.07 ± 0.01 [a]	0.09±0.01 [a]
Sodium chloride, %	0.41 ± 0.09 [a]	0.04 ± 0.01 [b]
Protein content, %	41.33 ± 0.11 [a]	43.38 ± 0.24 [b]
Sugars, %	18.47 ± 0.10 [a]	11.32 ± 0.21 [b]
Total dietary fibers, %	23.94±1.53 [a]	21.18±1.21 [a]
Calcium, mg/kg	5960 ± 0.22 [a]	6742 ± 0.32 [b]
Magnesium, mg/kg	1377 ± 0.12 [a]	1221 ± 0.42 [b]
Iron, mg/kg	1.44 ± 0.05 [a]	0.86 ± 0.03 [b]
Copper, mg/kg	4.40 ± 0.07 [a]	2.13 ± 0.06 [b]
Manganese, mg/kg	5.20 ± 0.13 [a]	3.38 ± 0.24 [b]

[a, b] – different letters signify statistical significance.

Table 4. Individual amino acids in the brewer's yeasts

Amino acid, g/100 g product	Brewer's yeast	
	Pasteurized	Non-pasteurized
Valine	1.22 ± 0.06 [a]	1.58 ± 0.07 [a]
Isoleucine	0.97 ± 0.11 [a]	1.15 ± 0.13 [a]
Leucine	0.33 ± 0.09 [a]	0.45 ± 0.08 [a]
Lysine	3.86 ± 0.17 [a]	4.97 ± 0.13 [b]
Methionine	0.55 ± 0.13 [a]	0.62 ± 0.11 [a]
Cysteine	0.48 ± 0.16 [a]	0.59 ± 0.17 [a]
Threonine	1.94 ± 0.09 [a]	2.14 ± 0.11 [a]
Tyrosine	2.04 ± 0.09 [a]	2.21 ± 0.09 [a]
Phenylalanine	2.02 ± 0.08 [a]	2.47 ± 0.09 [b]
Alanine	5.38 ± 0.07 [a]	5.86 ± 0.06 [b]
Arginine	2.50 ± 0.06 [a]	2.43 ± 0.05 [a]
Aspartic acid	2.04 ± 0.12 [a]	2.15 ± 0.14 [a]
Glycine	0.98 ± 0.12 [a]	1.53 ± 0.12 [b]
Proline	2.46 ± 0.10 [a]	2.66 ± 0.18 [a]
Serine	6.65 ± 0.08 [a]	6.96 ± 0.10 [a]
Hydroxyproline	2.46 ± 0.16 [a]	2.13 ± 0.06 [a]
Hystidine	5.08 ± 0.12 [a]	5.19 ± 0.14 [a]

[a, b] – different letters signify statistical significance.

In the subsequent analysis the individual amino acids building the proteins in the brewer's yeasts were determined by HPLC and the results are presented in Table 4. The results from the analysis demonstrated that both yeasts have similar amounts of amino acids with slightly higher content for the non-pasteurized brewer's yeast. Between the indispensable amino acids, lysine and tyrosine appeared in the highest quantities.

Furthermore, brewer's yeasts alone and in combination with water extract of *Rosa damascena* waste were investigated for ability to synthesize AgNPs. The results of the visual observation and UV-Vis studies are presented in Figs. 1 and 2, respectively.

The synthesis of AgNPs by water extract of *Rosa damascena* was fast – a darkening of the solution was observed between the 3rd and 4th minute (the UV-Vis studies confirmed this observation as the absorption increased – Fig. 2). For samples 3B and 4B with combinations of water extract of *Rosa damascena* and non-pasteurized and pasteurized brewer's yeasts formation of AgNPs after the 5th minute was also observed (both visually and by UV-Vis). For sample 1B (pasteurized brewer's yeast) no synthesis of AgNPS was observed; sample 2B was able to produce nanoparticles around 1 hour after the beginning of the reaction. This observations with the results in Table 2 for polyphenol content revealed that the polyphenolic compounds were among the main reducing agents. Nevertheless, the fact that non-pasteurized brewer's yeast was able to produce AgNPs showed that sugars, polysaccharides and proteins also played a role in the process of synthesis, although the synthesis has taken place relatively slower.

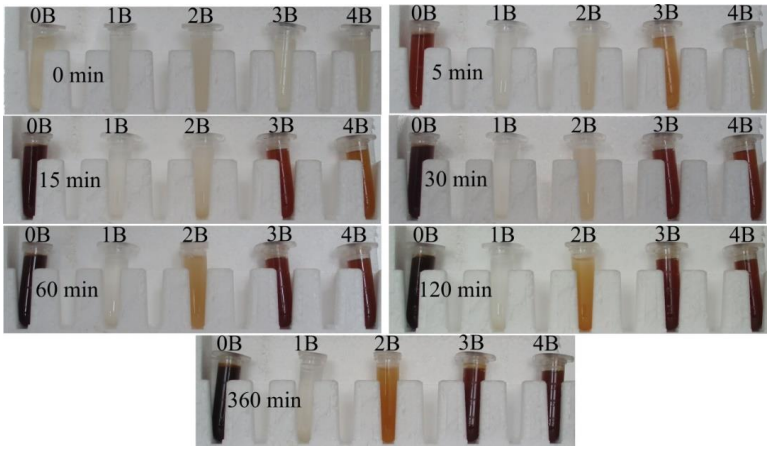

Figure 1. Visual observation of AgNPs synthesis. The sample numbers are according to Table 1.

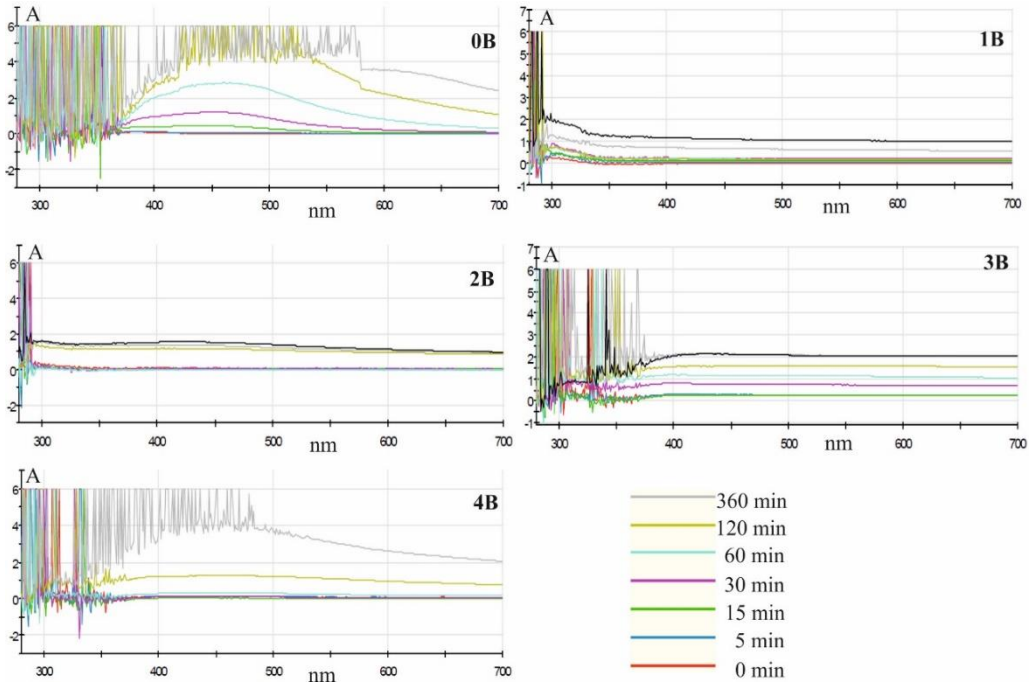

Figure 2. UV-Vis studies of the formation of AgNPs with time. The sample numbers are according to Table 1.

The TEM analysis confirmed the formation of nanoparticles with spherical shapes. Selective Area Electron Diffraction (SAED) results proved the AgNPs synthesis (Phase identified: Ag cubic, a=4.077, PDF 87-0720) – Fig. 3.

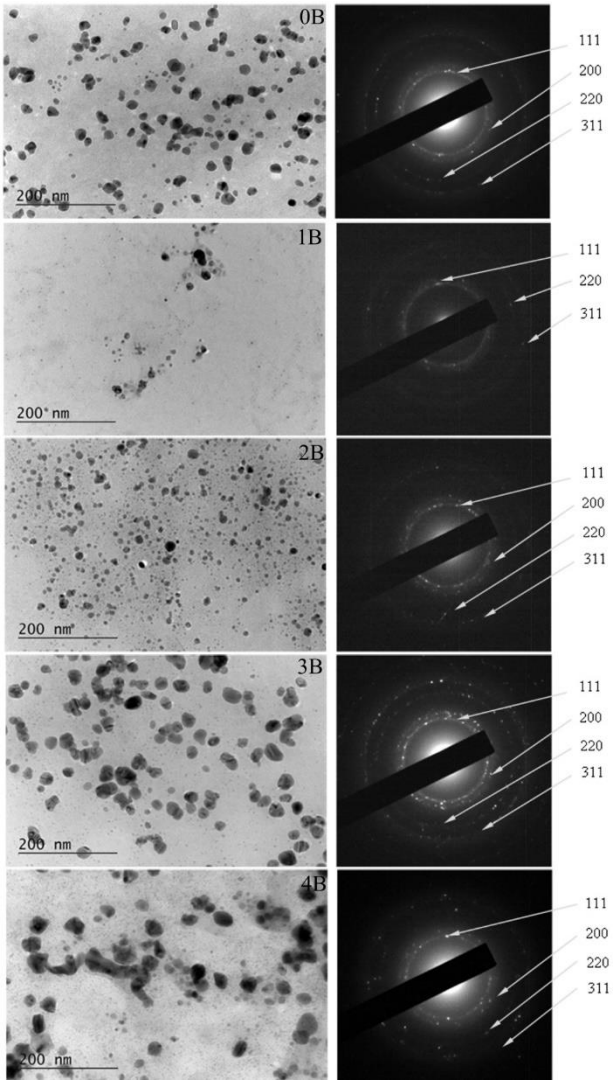

Figure 3. TEM analysis of AgNPs. The sample numbers are according to Table 1.

CONCLUSIONS

The present study focused on the characterization of brewer's yeast and its potential application (alone or in combination with water extract of *Rosa damascena* waste biomass) for "green" synthesis of AgNPs. The brewer's yeast was found to be rich in carbohydrates (polysaccharides and sugars), proteins and dietary fibers while having lower amounts of lipids which make them an excellent source for obtaining of functional foods. The water extract of *Rosa damascena*, rich in polyphenolic substances, was able to synthesize AgNPs quickly while both brewer's yeasts, owing to lower amounts of polyphenols, led to obtaining of silver nanoparticles with a significant delay in time. This slower process however, contributes to the aggregation of AgNPs and to a certain extent it could be beneficial for the final size and distribution pattern of the nanoparticles.

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