ORIGINAL PAPER

SILVER NANOPARTICLES BIOSYNTHESIS IN CROP EXTRACTS

ROXANA VLADOIU[1], RODICA-MARIANA ION[1,2*], SOFIA TEODORESCU[3], RALUCA MARIA STIRBESCU[3], IOANA-DANIELA DULAMA[3]

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Abstract. *The regular wheat and Spelta wheat are the most commonly used grains in human existence. In this paper, wheat and Spelta bran extracts were used $AgNO_3$ was added in order to obtain silver nanoparticles (AgNPs). For each type of bran, vitamins content (ascorbic acid, niacin, pyridoxine, thiamine, riboflavin) were determined by HPLC, in order to evaluate qualitative and quantitative the vitamins concentrations. FTIR, Raman and SEM-EDS techniques were used to characterize the extracts with $AgNO_3$, and AgNPs generated in-situ.*

Keywords: *wheat bran, Spelta bran, vitamins, Ag nanoparticles.*

1. INTRODUCTION

Over the past decades, the trend of miniaturization and the need to modernize technological processes increased and area like synthesis and properties of Ag nanoparticles raised a high interest in many areas. The studies conducted in 1980-1990 have shown that Ag nanoparticles have a rare combination of properties, unique optical properties associated with surface plasmon resonance, well-developed surfaces, catalytic activity, good electrical capacity of the double electric layer, etc. Also, colloidal Ag antibacterial agents (e.g., Colargol) have been shown to be very effective in medicine [1].

Currently, there are several ways of synthesizing silver nanoparticles by chemical, physical, photochemical and biological methods. Each method has its advantages and disadvantages, the main issues being cost, scalability, practice size and size distribution. The biological method offers many resources for the synthesis of silver nanoparticles and this method can be considered as an environmentally friendly approach and also a low-cost method. The reduction rate of metal ions by using biological agents is higher and occurs at ambient temperature and pressure. In biological synthesis, cell walls play a major role in the intracellular synthesis of the nanoparticles. The negatively charged cell wall interacts electrostatically with positively charged metal ions and reduces the metallic ions biologically to the nanoparticles [2].

When silver nanoparticles are produced by chemical synthesis, three main components are required: a silver salt (usually $AgNO_3$), a reducing agent (e.g., ethylene glycol) and a stabilizer or coating agent (e.g., PVP) in order to control the growth of nanoparticles and prevent their aggregation by collision. In the case of biological synthesis of silver

[1] Valahia University of Targoviste, Doctoral School of Materials Engineering, 130105 Targoviste, Romania. E-mail: rodica_ion2000@yahoo.co.uk
[2] National Institute for Research and Development in Chemistry and Petrochemistry - ICECHIM, 060021 Bucharest, Romania.
[3] Valahia University of Targoviste, Institute of Multidisciplinary Research for Science and Technology, 130004 Targoviste, Romania. **Corresponding Author*: rodica_ion2000@yahoo.co.uk

nanoparticles, the reducing agent and stabilizer are replaced by molecules produced by living organisms. These reducing and / or stabilizing compounds can be taken from bacteria, fungi, yeasts, algae or plants [3].

Plants and their parts contain carbohydrates, fats, proteins, nucleic acids, pigments and several types of secondary metabolites which act as reducing agents to produce nanoparticles from metal salts without producing any toxic by-product. Also, biomolecules such as enzymes, proteins and bio-surfactants present in microorganisms serve as reducing agents,and some biopolymers (e.g., starch, cellulose, chitosan, tree gum polymers), and other natural compounds like vitamins, proteins, peptides (e.g., glutathione), and sugars (e.g., glucose, fructose) are such materials, which provide suitable reducing and surface agents for the nanoparticle synthesis/stabilization [4-7].

Plant extracts are regarded as one of the most promising natural reducing agents, such as metabolites (e.g., sugars, alkaloids, polyphenols, phenolic acids terpenoids), and proteins and co-enzymes help to synthesis metal and metal oxide nanoparticles [8, 9]. These NPs can be used in biomedical applications due to their production advantages *via* biosynthetic route, which fashions the defined size, morphology and high chemical purity of NPs [10].

The biopolymers (cellulose and its derivatives, chitosan and its derivatives, alginate, dextran, and tree gums) are another family of natural sources used as reducing and stabilizing agents for metal and metal oxide nanoparticle synthesis [11-14].

Vitamin B1, Vitamin B2 (riboflavin), Vitamin C (ascorbic acid), coffee and tea extracts, beet juice, and grape pomace have been reported as natural reducing agents or antioxidants used for the synthesis of stable nanoparticles [15-17].

In this paper, the spectral, compositional and morphological characterizations of two wheat grains are investigated by FTIR, Raman and HPLC techniques. A method for simultaneous determination of water-soluble vitamins by HPLC with diode-array detection is reported. The proposed method is simple and rapid and it is possible to identify and simultaneously determine water-soluble vitamins in less than 25 min with only one injection. Also, the generation of silver nanoparticles in the presence of these crops extract is discussed, and their morphology is investigated by SEM-EDS.

2. MATERIALS AND METHODS

2.1. MATERIALS

2 mg of wheat bran and Spelta wheat bran have been prepared by grinding in the specialized mill. As reagent methanol (HPLC grade) and K_2HPO_4 (extra pure) from Merck, have been used. Water used in all the experiments was doubled distilled. The vitamin standards (ascorbic acid, niacin, pyridoxine, thiamine, and riboflavin) were of analytical-reagent grade from Agilent and were not further purified. Stock and standard solutions of water-soluble vitamins were prepared in the mobile phase. Five to seven different concentrations of each standard were used to prepare the calibration plot. These solutions were stored in dark glass flasks, to protect them from light, and kept under refrigeration. A calibration plot was prepared for each vitamin. Correlation coefficients for ascorbic acid, niacin, pyridoxine, thiamine, and riboflavin on the basis of plots of concentration ($\mu g\ mL^{-1}$) against peak area (mAU) were found to be >0.999.

2.2. METHODS

FT-IR investigations have been achieved using Vertex 80 FT-IR spectrometer, equipped with ATR device. The standard configuration is designed for data acquisition in the middle IR region (8000 to 350 cm^{-1}); the ATR-FTIR spectrometer uses dry air in order to reduce the content of unwanted atmospheric interferents.

For **Raman spectrometry**, Rigaku's Xantus-2 Raman Analyzer has been used for quickly and accurately identification of chemical substances based on the chemical fingerprint at the molecular level and is therefore highly specific.

The morphological characterization of AgNPs were achieved using **scanning electron microscope** (SEM) SU-70 by Hitachi, performed under 30 kV accelerating voltage and 16 mm working distance range, coupled with UltraDry EDS (Thermo Scientific).

Liquid chromatography was performed with an Agilent 1100 system consisting of: column C18, UV-visible diode-array detector, degasser, and liquid chromatography pump; Agilent software was used to calculate peak areas. The sample amount injected into the HPLC system was 20 µL.

A reversed-phase high-performance liquid chromatographic (RP-HPLC) procedure has been developed for the determination of water-soluble vitamins (ascorbic acid, niacin, pyridoxine (vitamin B6), thiamine (vitamin B1), and riboflavin (vitamin B2). The water-soluble vitamins were analyzed by HPLC on a Zorbax Eclipse XDB C18, 150 mm × 4.6 mm x 5µ column with 0.1 mol L^{-1} KH_2PO_4 (pH 7.0) – methanol, 90:10, as mobile phase (0.7 mL min^{-1}) in isocratic mode. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards stored in a data bank. The detection limits ranged from 0.1 to 0.5 mg L^{-1}. The column eluate was monitored with a photodiode-array detector at 265 nm for vitamin C, 234 nm for thiamine, 266 nm for riboflavin, 324 nm for pyridoxine, 204 nm for biotin, 261 nm for niacin. The mobile phase was filtered through a 0.45 µm membrane and degassed before use. The linearity of standard curves and detection limits for the water-soluble vitamins, Table 1.

Table 1. Linearity of standard curves and detection limits for the water-soluble vitamins.

Vitamin	Linear range (mg L^{-1})	R	r^2	Detection limit (mg L^{-1})
Ascorbic acid	5.0–200.0	0.9992	99.90	0.1
Niacin	10.0–200.0	0.9996	99.93	0.1
Pyridoxine	0.5–30.0	0.9990	99.82	0.1
Thiamine	1.0–50.0	0.9985	99.69	0.5
Riboflavin	1.0–40.0	0.9998	99.94	0.2

2.3. SAMPLE PREPARATION

Weight 2 g of sample (whole flour) in 50ml flacon, add 12mL of HCl 0.1N, stirr in a water bath at 70 °C for 10 min, centrifuge at 25 °C for 12 min at 4000 rpm, transfer 2 mL of supernatant in Eppendorf tube, centrifuge at 8500 rpm for 25 min, filter the solution, using RC filter 4 mm (0.45µm) in amber vials.

3. RESULTS AND DISCUSSION

Vitamins are a broad group of organic compounds that are minor, but essential, constituents of food required for normal growth, self-maintenance, and functioning of human and animal bodies. These compounds can be classified into two main groups – water-soluble and fat-soluble vitamins. Among the B group of water-soluble vitamins, both thiamine (B1) and pyridoxine (B6) are essential in human nutrition and their relative instability, qualitative and quantitative analyses are important issues and a challenging task for food manufacturers. HPLC is the preferred technique for vitamin separation, because of its high selectivity.

Table 2. Water-soluble vitamins content from wheat and spelta wheat.

Assay type	B1 (mg/100g)	B2 (mg/100g)	B3 (mg/100g)	B6 (mg/100g)	H (mg/100g)	C (mg/100g)
Wheat	1.770	4.735	2.481	0.117	97.00	2.942
Spelta wheat	1.701	4.707	2.544	0.179	83.72	9.723

As literature reported vitamin B1 effectively reduced Pd ions and the structures of the ensuing Pd nanoparticles could be well controlled as nanobelts, nanoplates, and nanotrees at different concentrations of palladium precursors [15]. Vitamin B2 was used as a reducing and capping agent for the synthesis of metal nanoparticles [16]. Because of its antioxidative properties, water-soluble vitamin B12 is used now in the present work to synthesize Ag, Au, and Pd metal nanoparticles [18, 19].

Taking into account these facts, the presence of different vitamins from wheat could be responsible for the biosynthesis of silver nanoparticles. First experiments have been achieved by FTIR, comparing both wheat types, as it is shown in Fig. 1. For the entire spectrum, no differences could be observed, so in principle these wheat sorts are identical.

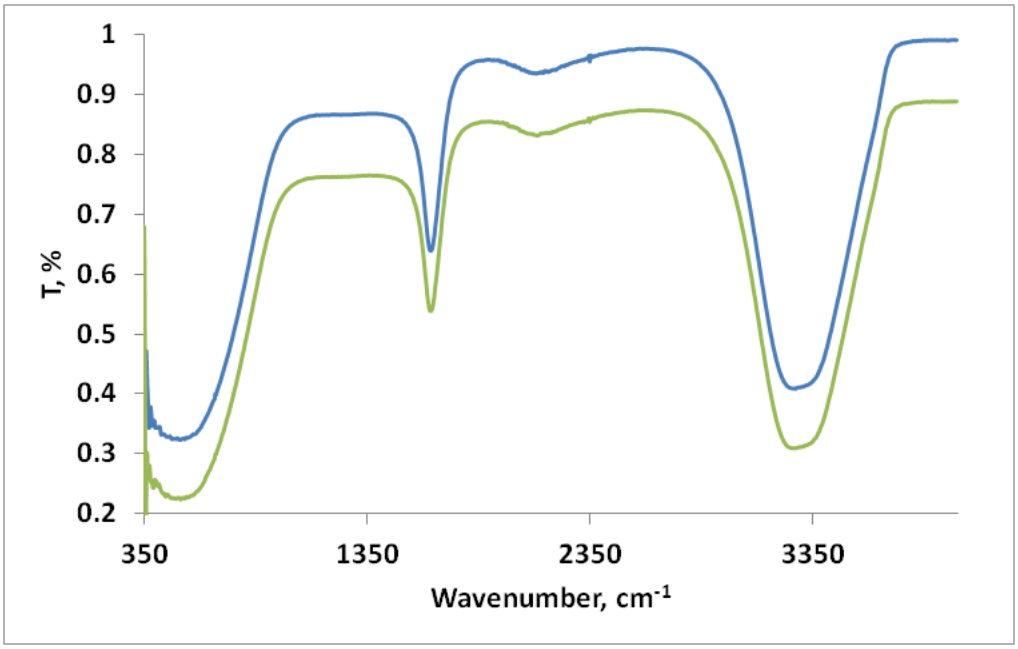

Figure 1. The spectrum of normal wheat (blue line) and spelta wheat (green line).

By analyzing the FTIR spectrum for both wheat bran types, the following aspects could be concluded: the broad absorption band between 3500-3000 cm^{-1} (3284.93 cm^{-1}), is attributed to the OH and NH range, while the peak at 1635 cm^{-1} corresponds to the absorption band of the protein, attributed to the C = O stretching vibration of the amide group present in proteins.

Meanwhile, a similar range 1690-1750 cm^{-1} is assigned to free carboxylic acid and to esters or to the carbonyl group of carboxylic aromatic acids generated by the oxidation of aliphatic chains or from vegetable oils, has been identified at 1715 cm^{-1} by Vasquez et al. [20].

For Spelta wheat bran similar results have been obtained. The 1444 cm^{-1} band to the deformation of CH bonds (CH_2 or CH_3 groups), the bands from 794 and 1001 cm^{-1}, could be assigned to phenolics and fatty acids, fatty acid ester, sitosterol and sitosterol acetate, have absorption in the region 500-200 cm^{-1} [21-26].

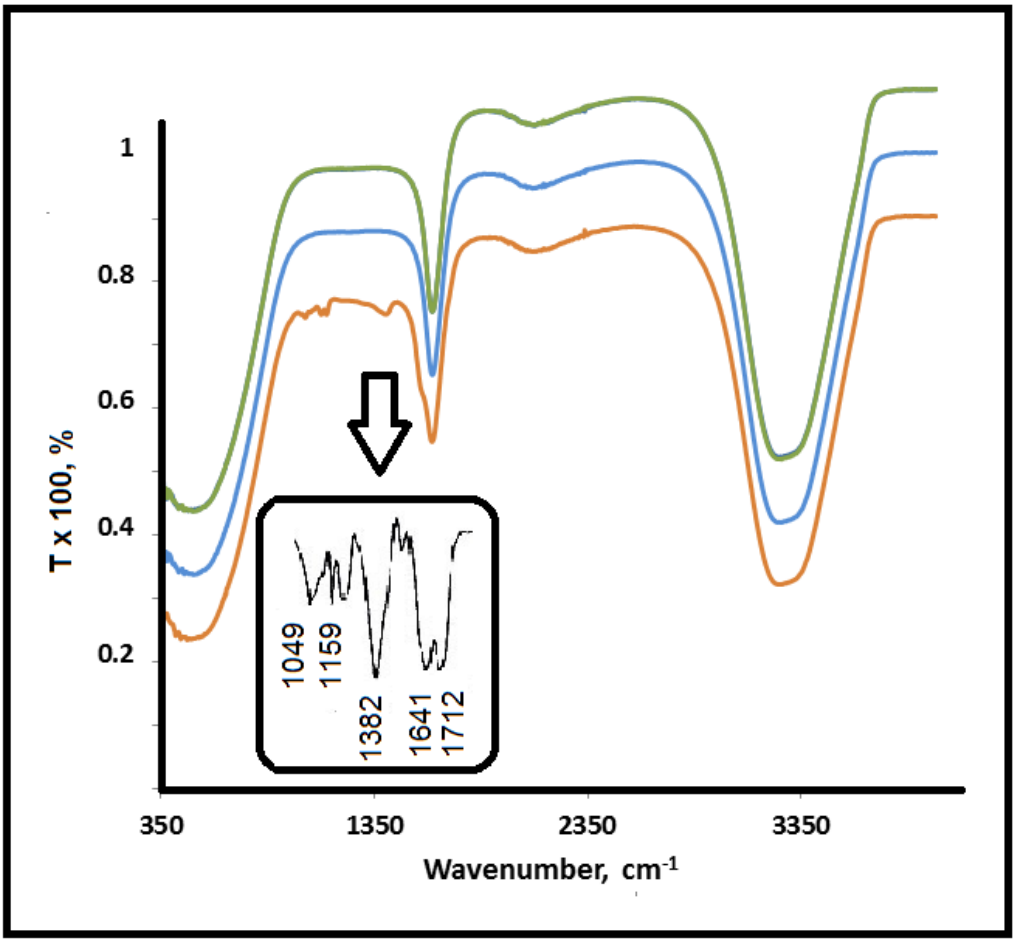

Figure 2. FTIR for Spelta wheat bran (green line), wheat extract with $AgNO_3$ (blue line), and wheat extract with $AgNO_3$ and vitamin C (orange line).

By mixing the cereal extract with $AgNO_3$ (0.5 mL filtered + 0.5 mL $AgNO_3$), was easy to obtain nanoparticles of silver, identified by FTIR and Raman spectroscopy. The reason is the hihgher concentration of Vitamin C (four time higher) than the regular wheat bran.

IR spectrum of Ag NPs generated in the presence of wheat extract (Fig. 2, orange line), shows absorption peaks at 1641 (shoulder), 1382, 1049 cm^{-1} in good agreement with literature [27].

In the Raman spectrum of both wheat types (Fig. 3), the peaks recorded at 1610, 1522 and 1448 cm^{-1} can be assigned to stretching vibrations C=C of phenyl ring of flavonoids. The carotenoid esters can be highlighted by the presence of peaks from 1752 cm^{-1}(stretching vibrations of carboxylic group C=O) and 1500 cm^{-1} (C-H symmetrical bending in $–CH_3$ or the carboxyl group COO). The peaks recorded at 961, 911, 860, 834, 518, 452, and 423 cm^{-1} corresponding to D-glucose compound. At 1060-1124 cm^{-1} C-C groups are present while at 1335-1411 cm^{-1} CH_3 groups are found.

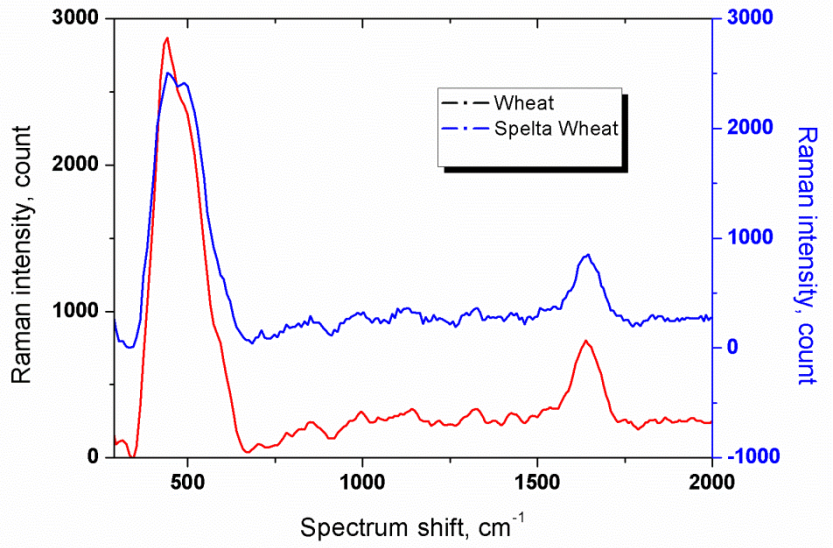

Figure 3. Raman spectra of regular wheat (red line) and spelta wheat (blue line).

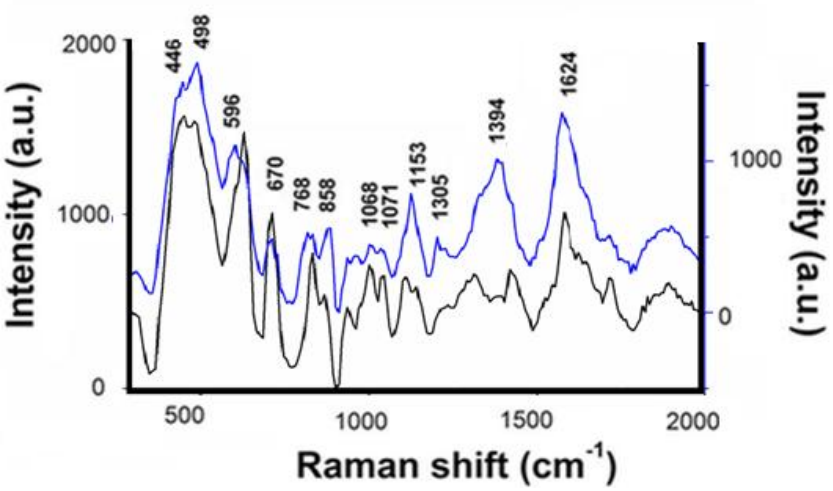

Figure 4. Raman Spectra: a) Spelta wheat extract with $AgNO_3$; b) Spelta wheat extract with $AgNO_3$ and vitamin C.

After silver nitrate addition, the spectrum shows a sharp band at 240 cm^{-1}, attributed to the stretching vibrations of Ag–N and Ag–O bonds [28], due to the chemical bonds present in the organic components of wheat. The bands at 1068, 1153 and 1305 cm^{-1}, and 768 and 858 cm^{-1}coming from the C–H in plane bending and out of plane wag, respectively from the saccharide structure of cereals. The bands from small wavenumbers (445 and 496; 670 and 1394 cm^{-1}assigned, respectively, to the stretching vibrations of (C–N–C), (C–S–C) and phenyl ring. Thus, from the preferential enhancement of these Raman bands; it can be concluded that both amino and carboxylate groups of the wheat are involved in silver nanoparticles generation. By SEM-EDS, was possible that from wheat extract with $AgNO_3$, to

put into evidence silver nanoparticles, either as spherical particles or as flower-shaped nanoparticles (Fig. 5).

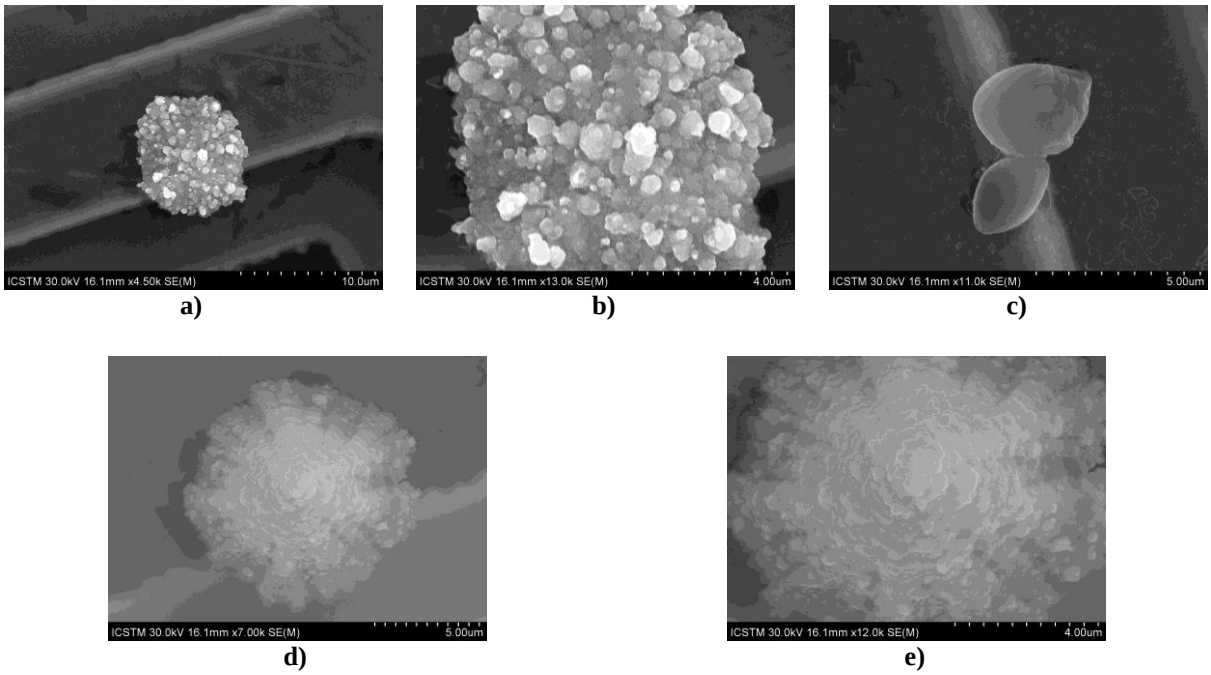

Figure 5. SEM image for wheat extract and $AgNO_3$: a) NPs agglomeration 4500x; b) NPs agglomeration 13000x;c) *Saccharomyces cerevisiae*, 11000x; d) NPs agglomeration 7000x; e) NPs agglomeration 12000x.

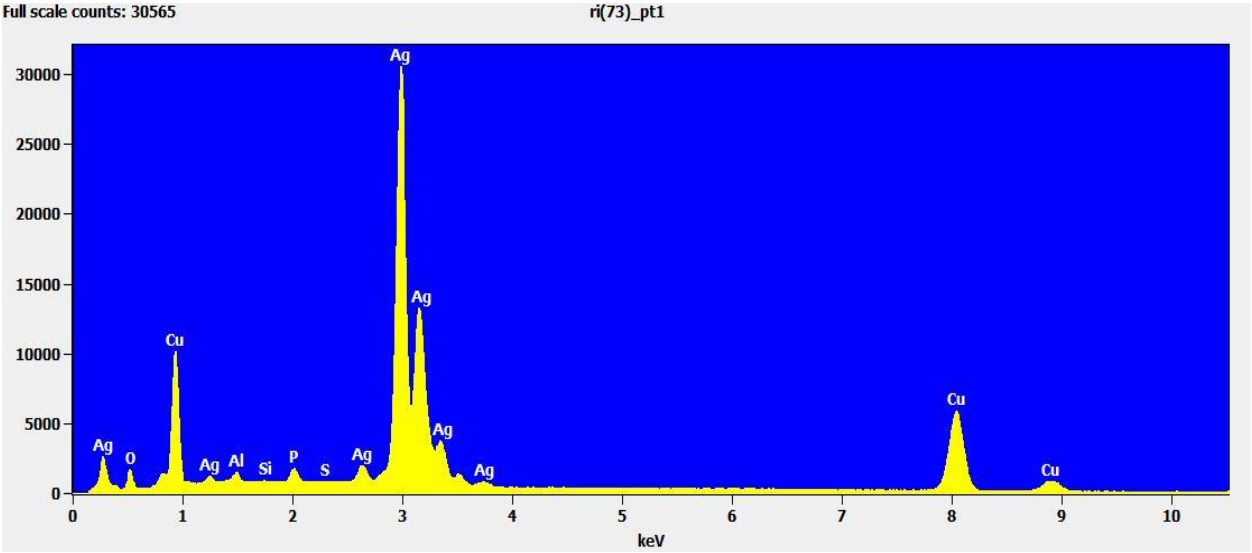

Figure 6. EDS for AgNPs from wheat extract with $AgNO_3$.

In Fig. 5 can be observed few structures resulted by nanoparticles agglomeration, as well as the presence of yeast (*Saccharomyces cerevisiae*) (Fig. 5c). The elemental content (Fig. 6) highlights the presence of silver, as well as Al, Si, P, S, O, Cu. These elements come from both wheat extracts (i.e. Si, P, S, and O) and sample support (i.e. Al and Cu).

The silver tips are present due to the X-ray signal from AgNO3, and the wheat extract contains inorganic compounds related to Ag. For Spelta wheat and $AgNO_3$, SEM-EDS results reveal similar results as previous, except the spatial orientation (star or flower-shape) of the associated nanoparticles (Fig. 7).

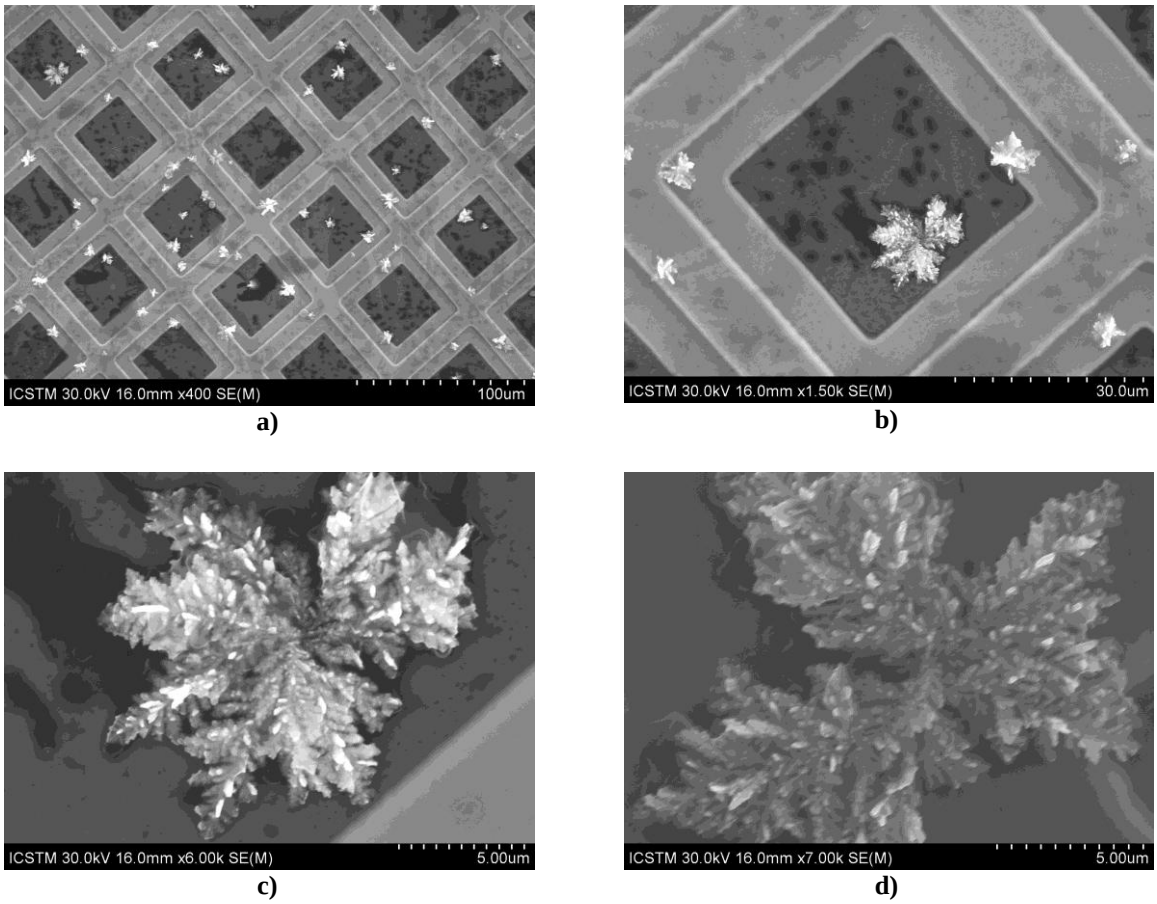

Figure 7. SEM image of Spelta wheat extract with $AgNO_3$: a) NPs agglomeration 400x; b) NPs agglomeration 1500x; c) NPs agglomeration 6000x; d) NPs agglomeration 7000x.

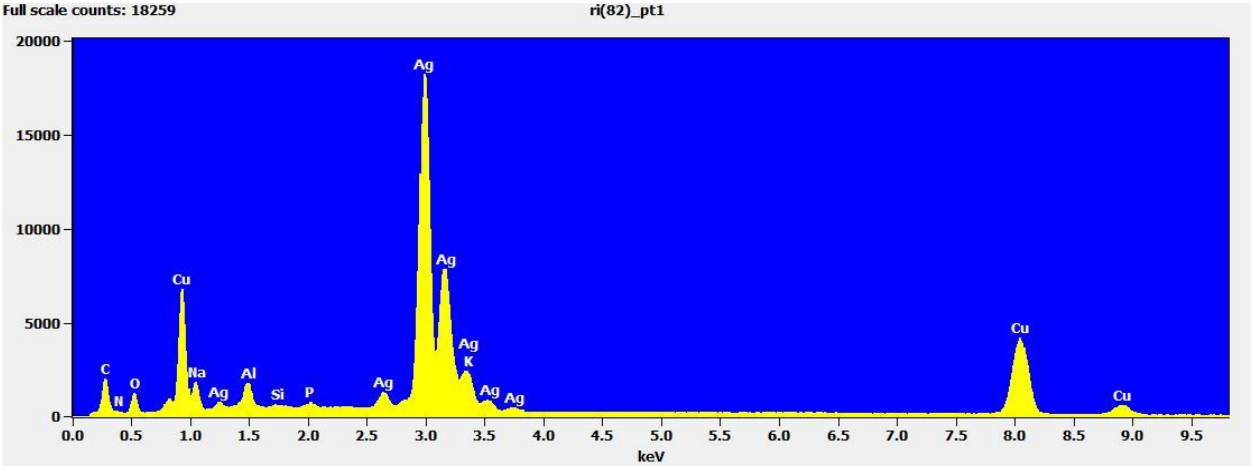

Figure 8. EDS for AgNPs from Spelta wheat extract with $AgNO_3$.

The EDS spectrum confirmed the presence of silver by the strong signal at 3 KeV (Fig. 8) obtained on Spelta extract with $AgNO_3$ are similar with the data obtained for wheat extract (Fig. 6).

4. CONCLUSION

The presence of vitamins B1, B2, B3, B6, H, and C was highlighted in the extracts obtained from regular wheat bran and Spelta bran. The bran extracts with $AgNO_3$ were analyzed by FTIR and Raman spectroscopy, comparative with initial bran extracts. The silver nanoparticles generate in bran extracts by $AgNO_3$ addition were highlighted by FTIR, Raman and SEM-EDS analysis. The Spelta wheat bran extract is more adequate for AgNPs generation, due to its higher concentration of Vitamin C, which is recognized as an efficient reducer agent. In regular wheat bran extract, the generated AgNPs is more spherical or flower-shape, while in Spelta wheat bran extract, the nanoparticles are star or flower-shaped.

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