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# Immobilization of natural betalain pigments in inorganic hosts

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*Abstract*: In search of new food-grade pH sensitive formulations, red beet extract rich in betacyanin was included in different inorganic matrices based on silica and aluminosilicate to improve the stability of the dye. By the direct method of encapsulation of the pigments in silica support, stabilizing agents such as inclusion complex forming *β*-cyclodextrin and ascorbic acid were added. The post loading system assumes the synthesis of porous silica and aluminosilicate powders and the adsorption of the beet extract by these supports. The unloaded carriers were structurally and texturally characterized (X-ray diffraction, FTIR, $N_2$-physisorption). The presence of betanin, approved as a red food colorant, was evidenced by UV–Vis spectroscopy in all the hosts. Color properties were investigated as well as the pH generated color variations of the powders exposed to ammonia in the head-space of a sealed vessel. The obtained results could widen the field of applications of beet extract, the prepared ecological formulations could provide added value to edible products packaging.

*Keywords*: betanin; encapsulation; mesoporous silica; aluminosilicate; color parameters.

## INTRODUCTION

Betalains are the major pigments abundantly found in beetroot (*Beta vulgaris)* which are chemically defined as the derivatives of betalamic acid.[1] Betalains can be divided into yellow-orange betaxanthins and red-violet betacyanins. Of the betalains, betanin is approved for use as colorant in food industry (E number E162, CI Natural Red 33) and is considered a better choice than chemically synthesized pigments. It has commercial applications for foodstuff, cosmetics and pharmaceuticals.[2] Betanin is almost totally extracted from beetroot

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crops and sold as beetroot extract.[3] All betalains are water soluble. The main factors influencing their stability are light, oxygen concentration, temperature, the presence of water and pH. They retain their tinctorial strength and color shade over a pH range of 3–7.[1,2] The fact that betacyanins show sensitivity to pH makes them suitable for use as colorimetric indicators for detecting changes of the environment especially in case of edible products, thus improving the protection and shelf life of foodstuffs or pharmaceuticals.[4] To preserve the chemical structure of the pigment and to facilitate its use in commercial applications, different ways of stabilization were used. Metal–chelates formation, cyclodextrin complexes, addition of organic acids, inclusion in different matrices should be mentioned.[5,6] It was shown that cyclodextrins are promising stabilizing agents of betalain molecules, $\beta$-cyclodextrin ($\beta$-CD) forming an inclusion complex with betanin.[5,6] Copigmentation was used to preserve betalains color intensity and stability. It involves interactions between the dyes and other naturally occurring compounds such as ascorbic or citric acid which can be neutralizing agents for the electrophilic center of betalains.[5,6]

Immobilization of the beet pigments on a solid carrier, organic or inorganic, is a promising approach for improving the properties of the plant derived pigments, thus enabling the inclusion of certain additives that can confer better functionality to encapsulated betalain pigments. A wide range of encapsulation systems have been designed: natural products derived nanocarriers (maltodextrin, caseins), lipid-based nanocarriers, biopolymers based nanocarriers (chitosan, proteins) and inorganic carriers (carbon nanotubes, clay minerals, $\gamma$-alumina).[6–8] Two approaches for the inclusion of drugs into silica based particles of matrix type have been mentioned, namely direct drug encapsulation when the drug is dissolved in the liquid precursor prior to formation of the matrix and indirect encapsulation or post loading when carrier particles are first synthesized followed by encapsulation of the drug.[9,10]

The purpose of the present work was to obtain formulations betalains–$SiO_2$ and betalains-aluminosilicate, respectively, and to explore the two above mentioned loading modes of beet pigments into the silica-based carriers. Our results provide novel information about the structure and properties of the final hybrid pigments, especially the color response toward ammonia, a marker of food spoilage, the obtained data contributing to the development of product formulations which can favour the properties of food packaging.

## EXPERIMENTAL

### *Materials*

Ludox®SM-30 colloidal silica (30 wt. % suspension in water) and sodium hydroxide were from Sigma Aldrich. $\beta$-cyclodextrine was purchased from Wacker-Chemie GmbH. Ascorbic acid pharmaceutical grade was purchased from Intra-Laboratories, cetyltrimethylammonium bromide (CTAB) from Carl Roth, aluminium oxide from Merck Millipore, sodium dodecyl sulfate (SDS) analytical grade from Applichem. The concentrated beetroot juice (100 %, Biotta food grade), produced by extraction of pigments from red beet and its subsequent pasteurization with betanin the major coloring principle, was used as natural dye.

### *Characterization*

X-ray diffraction (XRD) analyses were performed using a 9 kW Rigaku SmartLab diffractometer (Rigaku Corp., Tokyo, Japan, operated at 45 kV and 200 mA, CuK$\alpha$ radiation $\lambda$ =1.54059 Å), in scanning mode $2\theta/\theta$, between 2 and 90° ($2\theta$) for wide-angle analysis and between 0.05 and 8° ($2\theta$) for small angle analysis, measuring scan step 0.02°, scan speed 8° min$^{-1}$ (wide angle) and 4° min$^{-1}$ (small angle). Components were identified by comparison with ICDD data. FT-IR spectra were recorded on a Brucker Vertex 70 spectrometer with horizontal device for attenuated reflectance and diamond crystal, on a spectral window ranging from 4000 to 400 cm$^{-1}$, at a spectral resolution of 2 cm$^{-1}$. Spectra were recorded without any sample preparation and were processed with OPUS 5.5 program (Brucker). Nitrogen adsorption–desorption isotherms at 77 K were recorded on a Micromeritics ASAP 2020 automated gas adsorption system (Norcross, GA, USA). The samples were degassed at 250 °C for 4 h under vacuum before analysis. Specific surface areas ($S_{\rm BET}$) were calculated according to the Brunauer–Emmett–Teller (BET) equation, using adsorption data in the relative pressure range between 0.05 and 0.30. The total pore volume ($V_{\rm total}$) was estimated from the amount adsorbed at the relative pressure of 0.99. The pore size distribution curves were obtained from the desorption data using the Barrett–Joyner–Halenda (BJH) model. UV–Vis spectra were recorded using a Jasco V-750 spectrophotometer equipped with an integrating sphere that allows measurements on solid samples. Chromatic parameters were registered with a Konica Minolta CR-410 colorimeter with a white calibration plate ($Y$ = 94.27, $x$ = -1.06, $y$ = 3.45), measurements were done in triplicate and means reported. In the CIE $L^*a^*b^*$ uniform color space the color coordinates are: $L^*$ (brightness), $a^*$ (green-red) chromaticity, $b^*$ (blue–yellow) chromaticity to describe the color of the matrices.[11]

### *Preparation*

The two approaches, mentioned above, were used to include beet extract into the carriers. In the direct encapsulation procedure an aqueous mixture of 5 ml beet juice and 0.1 g $\beta$-CD is added, under constant stirring and room temperature, to 20 ml colloidal silica which was previously diluted with 12 ml water and brought to a pH of ~4 with an ascorbic acid solution of 15 %. Then the dye containing composition was introduced in a vacuum rotary evaporator and kept at 30 °C under vacuum until the liquid was evaporated and the precipitate was separated and crushed into powder. It was denoted silica-bet 1.

Two mesoporous supports were prepared for beet juice adsorption. One of them, silica 2, was obtained from colloidal silica and CTAB as template agent, the molar ratio $SiO_2$/CTAB being equal to $0.12/0.3\times10^{-3}$. The mixture was brought to an acid pH with an acetic acid solution 20 %, under stirring and kept for 5 days at room temperature. The other one, an aluminosilicate carrier (silicaAl 3) was obtained by a hydrothermal process (90 °C, 48 h) in a basic medium from colloidal silica and $Na_2O\cdot Al_2O_3$ using SDS as a structure directing surfactant. The molar ratios of the components were $SiO_2/Al_2O_3$ = 6, $SiO_2$ /$Na_2O$ = 10/7, $SiO_2$/SDS = 10/1.4. The silica 2 and silicaAl 3 powders were separated by repeated centrifugation and washing with distilled water and then they were thermally treated at 600 °C, with 5 h plateau and a heating rate of 1 °C min$^{-1}$. 0.8 g of each host material was mixed with 1.2 ml beet juice, stirred for homogenization and followed by 5 h vacuum drying in dark conditions. A colored powder was obtained.

Equal quantities of the three dye loaded powders were spread on plates and were put in a PET container together with a Petri dish containing ammonium hydroxide solution 25 %. The container was sealed for about 15 min so that the powders should be in contact with the ammonium vapours developed into the headspace. The color changes were determined.

## RESULTS AND DISCUSSION

### *Structural characterization of the unloaded matrices*

*X-Ray diffraction*. The crystallographic structure of the as prepared samples was evaluated by XRD analysis. The diffraction patterns of the powders are presented in Fig. 1. As can be seen in Fig. 1a, the diffraction patterns indicate that the silica carrier is amorphous, marked by the existence of a broad peak at $2\theta$ in

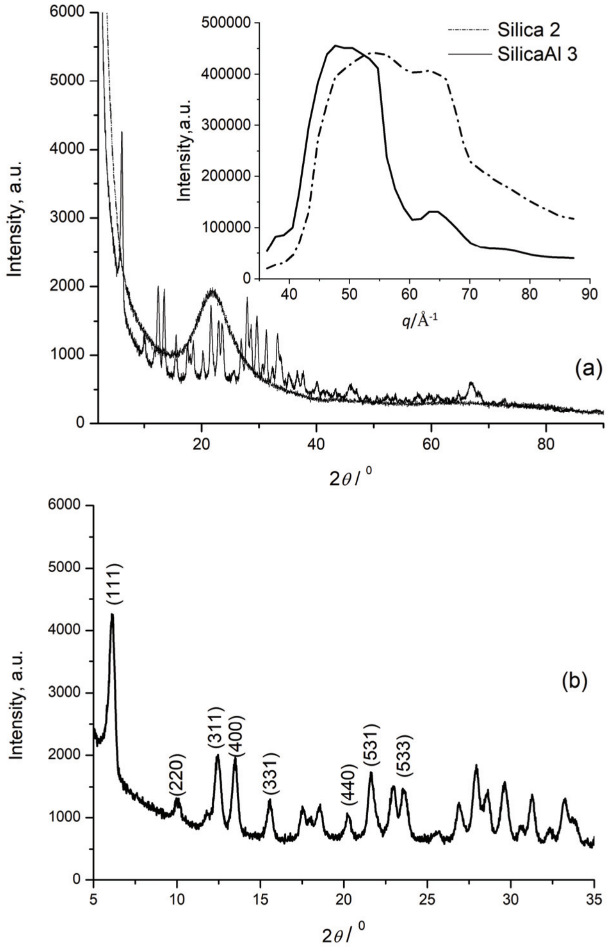

Fig. 1. XRD patterns of Silica 2 and silicaAl 3 (the inset shows SAXS diagram of the same unloaded carriers) (a) and details of crystal structure of silicaAl 3 (b).

the range 20–35° attributed to amorphous silica.[12] The pattern of the aluminosilicate sample in Fig. 1b shows the formation of a crystalline phase of the zeolite Y type (identification was performed using PDXL software from Rigaku connected to ICDD database).

The inset of Fig. 1a shows the SAXS diagram of the two as prepared powders. The variation of the scattering intensities with the scattering vector (*q*) is due to the electron density difference between the matrices and the empty pores.[13,14] The scattering curves of each of the two samples present a regular structure, confirming the long-range well-ordered mesostructured.[15,16]

*$N_2$ physisorption*. In Fig. 2 one can see that both samples show type IV isotherms according to IUPAC classification, typical for mesoporous materials.

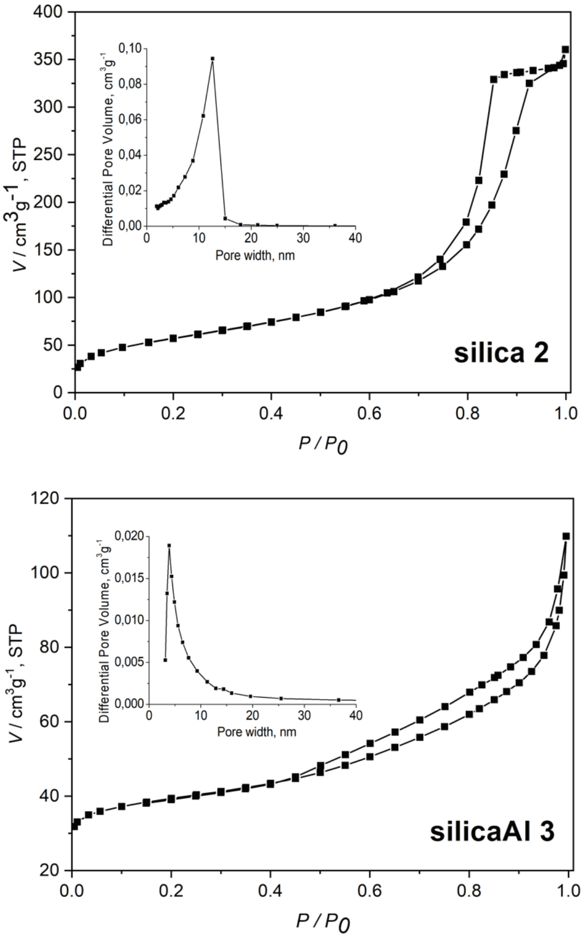

Fig. 2. $N_2$ adsorption–desorption isotherms and pore size distributions (inset of the figures) of silica 2 and silicaAl 3 supports.

Silica 2 support exhibits a H2 hysteresis loop that indicate the existence of a complex pore structure in which network effects are important. In the case of silicaAl 3 support, the hysteresis loop is of H3 type suggesting an assemblage of slit-shaped pores. The pore size distribution graphs show a unimodal distribution for both types of supports with peak maxima at 12.6 nm for silica and 3.8 nm for aluminosilicate. A *t*-plot analysis indicated the absence of microporosity in the silica support, whereas in aluminosilicate an important fraction of micropores was detected (~70 %). BET surface area, as well as total pore volume are higher for silica type carrier than for zeolite-Y type aluminosilicate (Table I).

TABLE I. Textural parameters of silica 2 and silicaAl 3 supports

| Sample | $S_{\mathrm{BET}}$ / m$^2$ g$^{-1}$ | $V_{\mathrm{total}}$ / cm$^3$ g$^{-1}$ |
|---|---|---|
| Silica 2 | 205.3 | 0.557 |
| silicaAl3 | 148.5 | 0.138 |

Porosity measurements as well as the X-ray analysis prove that the supports are highly ordered materials.

*Infrared absorption spectra*. Fig. 3 shows the FTIR spectra of the unloaded matrices. The strongest absorption bands of the two samples lie in the wavenumbers range 1200–950 cm$^{-1}$ described as an asymmetric stretching mode O–(Si,Al)–O.[17] Silica 2 sample shows a peak at 1110 cm$^{-1}$, while silicaAl 3 at 986 cm$^{-1}$. The presence of Al atoms in the structure generates a shift to lower wavenumbers.[16]

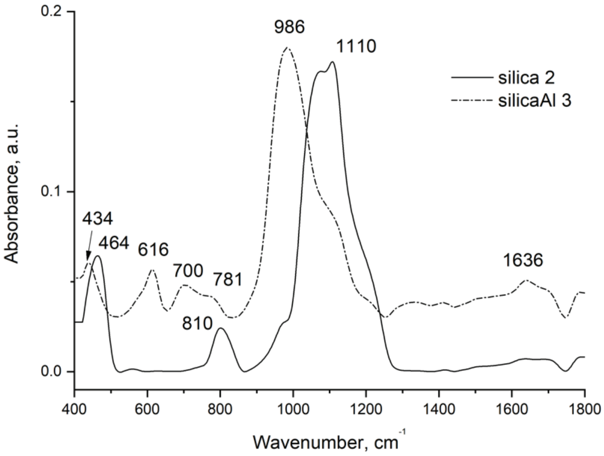

Fig 3. FTIR spectra of silica 2 and silicaAl 3 supports.

In the region 420–500 cm$^{-1}$ the bands are assigned to O–(Si, Al)–O bending mode.[17] A strong sharp band can be seen in the spectrum of silica 2 matrix at 464 cm$^{-1}$. In the aluminum containing one it is shifted to 434 cm$^{-1}$ and becomes weaker. Bands corresponding to symmetric stretching modes appear in the range 650–820 cm$^{-1}$.[17] In the spectrum of the silica 2 sample a single band at 810 cm$^{-1}$ can be attributed to Si–O–Si sym stretching,[18] while the presence of Al in the sample generates a widening and flattening of the bands (781 and 700 cm$^{-1}$).[18] The absorption bands at 1636 cm$^{-1}$ show the presence of water in the matrices.[12]

FTIR spectra confirm the formation of an aluminosilicate type framework in accordance with XRD results and show that the structure directing agent was removed by thermal treatment.

*Characterization of matrices loaded with beet juice pigment*

*UV–Vis spectroscopy of the dye-carrier formulation*. There is an electron resonance system based on the two nitrogen atoms of the betalains molecules which is linked to the colour properties.[19] Bethaxanthins with a more simple structure around nitrogen atom have a typical UV–Vis absorption maximum in the 457–480 nm region and are yellow while the betacyanins with an extended resonance system generate wavelengths in the range 524–542 nm that makes them violet.[19–22]

Fig. 4 presents the UV–Vis spectra of the three prepared samples by inclusion and adsoption. All display the betanin absorption maximum (535–543 nm). Compared to silica-bet 1 spectrum, in which case the pigments can be stabilized by inclusion complex formation with *β*-CD,[5] the silica 2 and silicaAl 3 samples have different profiles. In case of silica 2, an overlapping of the bands is observed in the 472–535 nm range and silicaAl 3 spectrum shows shifts to lower (415 nm) and higher values (543 nm). Betalains are known as electron donating compounds,[21] and this fact can be due to interactions with the matrices which

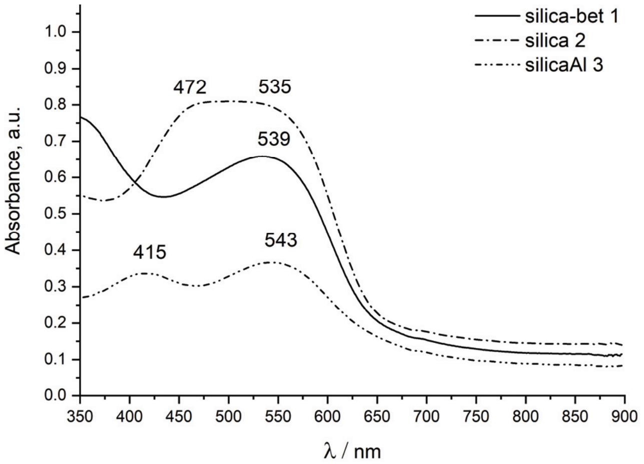

Fig. 4. UV–Vis spectra of the beetroot dye formulations based on silica and aluminosilicate.

become more significant for a high specific surface area and micro/mesoporosity.[23,24] Aluminium coordinative unsaturated sites in the network of silicaAl 3 enhances the interactions with adsorbed betalains.[8,24]

*Effect of $NH_3$ vapors on dye-carrier formulations color change.* Mean $L^*$, $a^*$ and $b^*$ values for the dye loaded powders before and after ammonium exposure are presented in Table II.

TABLE II. Color parameters for beet juice in different matrices before and after exposure to base atmosphere; data are presented as mean ± standard deviation of triplicate analyses

| Sample | Color parameters before exposure | | | Color parameters in $NH_3$ atmosphere | | | $\Delta E$ |
|---|---|---|---|---|---|---|---|
| | $L^*$ | $a^*$ | $b^*$ | $L^*$ | $a^*$ | $b^*$ | |
| Silica 2 | 84.78±0.43 | 4.49±0.17 | –1.16±0.033 | 83.13 ±0.29 | 3.60±0.14 | 0.70±0.089 | 1.87 |
| SilicaAl 3 | 86.21±0.35 | 3.69±0.38 | –1.78±0.014 | 86.34±0.11 | 1.99±0.05 | 0.25±0.080 | 2.65 |
| Silica-bet1 | 80.02±0.21 | 7.68±0.12 | –0.24±0.010 | 85.12±0.47 | 2.80±0.20 | 1.72±0.270 | 7.32 |

The other parameter in Table II, $\Delta E$, is the color difference between before ($L_0$, $a_0$, $b_0$) and after exposure to $NH_3$ ($L$, $a$, $B$) and was determined using the following equation:[25]

$$\Delta E = [(L_0 – L^*)^2 + (a_0 – a^*)^2 + (b_0 – b^*)^2]^{1/2} \tag{1}$$

The values of the color parameters presented above, indicate that the as prepared matrices containing beet juice are different: silica-bet 1 and silica 2 are redder, while silicaAl 3 is lighter and yellower. Values $\Delta E$ between 1.5–3 (silica 2 and silicaAl 3 sample) indicate distinct and over 6 (silica-bet 1 sample) great differences between the initially prepared samples and the ones exposed to ammonia.[26]

## CONCLUSION

Betalains pigments were included in silica and respectively aluminosilicate carriers to stabilize and improve their properties for practical applications. The direct encapsulation approach enabled the incorporation of other natural occurring compounds besides the dye, such as the complex forming *β*-CD and ascorbic acid, thus upgrading the prepared formulation regarding the color properties and the color variation with pH change. Structure directing agents generated mesoporous silica and zeolite *Y*-type supports with a complex, regular pore layout for the colorant adsorption. Betalains color properties depend on the variation in the physicochemical characteristics of the micro/mesoporous supports.

## ИЗВОД

# ИМОБИЛИЗАЦИЈА ПРИРОДНИХ БЕТАЛАИНСКИХ ПИГМЕНАТА У НЕОРГАНСКИМ МАТЕРИЈАЛИМА

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У циљу испитивању нових pH осетљивих формулација хране, екстракт црвене цвекле богат бетацијанином, ради побољшања стабилности његове боје, је наношен на различите неорганске подлоге које садрже силицијум-диоксид и алуминосиликате. Директном методом инкапсулације пигмента на неорганску подлогу која садржи силицијум-диоксид и алуминосиликат додат је стабилизатор који представља инклузиони комплекс $\beta$-циклодекстрина и аскорбинске киселине. Систем накнадног пуњења укључује синтезу порозног силицијум-диоксид и алуминосиликатног праха и адсорпцију екстракта репе на овим подлогама. Пре наношења екстракта извршена је структурна карактеризација неорганске подлоге (дифракција рендгенских зрака, FTIR и $N_2$-физисорпција). Присуство бетанина (одобрен као црвена боја за храну) у испитиваним узорцима је одређено применом UV–Vis спектроскопије. Испитиване су промене боје, као и pH генерисане промене боје прахова изложених дејству амонијака при врху капсулираних посуда. Добијени резултати могу допринети већој примени екстракта репе, док предложене еколошке формулације могу бити од значаја код паковања хране.

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