Decomposition of 17-decarboxy-betanin in selected aqueous-organic solutions induced by Cu (II) cations

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Abstract: The influence of aqueous-methanolic and aqueous-acetonic solutions on decomposition of 17-decarboxy-betanin in the presence of Cu (II) cations was compared at different pHs. The 17-decarboxy-betanin is a derivative of betanin, which is a natural red-violet betacyanin pigment sourced from red beet root for food industry application. These natural colorants have many advantages because of their safety as well as non-toxic and antioxidant properties. However, they are suggested to be structurally unstable in the presence of some factors, for instance at catalytic amounts of heavy metal cations. These compounds can be decomposed by contact with metal cations during manufacturing and storage processes. In this study, the effect of different concentrations of Cu (II) cations in selected solutions and pH on the pigment degradation was tested.

1. Introduction

17-decarboxy-betanin is a bright orange-red pigment, obtained as a derivative of betanin, a natural red-violet compound belonging to betacyanin pigments. The acidified solution of betanin subjected to heating at 85 °C results in the formation of decarboxylated derivatives with 17-decarboxy-betanin as the dominant product (Wybraniec, 2005). Structurally, betanin and its derivatives are one of the simplest betacyanins (Fig. 1).

Betacyanin pigments are responsible for color of many plants of the Caryophyllales order (Strack, 1993). Depending on the species, they are located in flowers, fruits, leaves or roots. One of the most popular sources of betanin is red beet root (Beta vulgaris L.). It is used to obtain betanin rich juice, and as a concentrate or dried powder it is used in food industry as a natural colorant.

Because betalain structures contain a phenolic group, from 1980s they have been considered to be able to stabilize radicals (Moreno, 2008). In the following years it...
was proved that betalains prevent active oxygen-induced and free radical-mediated oxidation. It was extensively tested in vitro on biological molecules as well as in vivo. The results showed protective antioxidant activity of betalains against oxidative stress on human low density lipoproteins, lipids of the cell membrane and red blood cells as well.

Nowadays, more and more studies prove that consuming food which contains antioxidants can prevent lots of serious diseases and delay the aging process. The significant issue is that antioxidants are very unstable compounds, sensitive to many factors causing their degradation and deactivation.

This is the reason why it is important to carry on investigations on betalain stability. The detrimental influence of high temperature, very acidic or basic pH, increased water activity, UV radiation and presence of several metal cations on betalains stability have already been reported (Herbach, 2006). However, many additional factors or their coincidence, related to manufacturing processes and storage have to be taken into consideration. The amount of natural antioxidants in food might be decreased because of heating during preparation process, exposure to light or oxygen in the air, or by contact with metal ions (e.g. from machine elements submitted for abrasion or metal containers like cans, where food is kept).

While conducting research on antioxidants in laboratory, one must be aware of the possible presence of metal cations in reagents or even in plant material. It is essential to check the influence of every factor and reagent which could come in contact with the tested compounds during preparation and experiment steps like: extraction, purification and analysis.

In this study, the impact of two solvents, aqueous-acetonic and aqueous-methanolic, (very frequently applied in chromatography) on the decomposition of 17-decarboxy-betanin resulting from the presence of copper cations is tested.

2. Materials and methods

17-decarboxylated-betanin was obtained by heating of betanin aqueous solution acidified with formic acid for 30 min at 85 °C. For this aim, betanin was isolated from red beet root juice (Beta vulgaris L.) and preliminarily purified by chromatographic methods.
2.1. Isolation of 17-decarboxy-betanin

The isolation of 17-decarboxy-betanin was carried out by a sequence of chromatographic techniques. First, purification using a C18 cartridge was carried out. The fractions were eluted by aqueous solution of 30 % acetone and 2 % formic acid (v/v). Next, flash chromatography was used with a gradient system: 5 % A in B at 0 min; gradient to 35 % A in B at 30 min (A, acetone; B, 2 % formic acid in water). Afterwards, a preparative HPLC system with HPLC PUMP 64 (Knauer, Germany) was applied. The semi-preparative column was Bischoff C18 (Bischoff Chromatography, Germany) 250 mm x 30 mm i.d., 10 µm, C18, with a 10 mm x 10 mm i.d. guard column with the same material. An applied gradient system was 6 % A in B at 0 min; gradient to 10 % A in B at 30 min (A, acetone; B, 1 % formic acid in water). In each case, the injection volume was 100 mL and the flow rate was 3 mL/min. The signal detection was performed at 505 nm by a UV-Vis detector (Knauer, Germany). The temperature during chromatographic isolation was ambient.

2.2. UV-Vis spectra measurement

The decomposition of 17-decarboxy-betanin was monitored by collecting UV-Vis spectra in the range of 350 – 550 nm after 2, 10 and 24 h during experiments using microplate reader Inffnite M200 (TECAN, Austria). The samples were tested in 96-well microplates, consisting of eight rows (A to H) and 12 columns (1 through 12). The influence of several factors on the detrimental effect of copper cations was studied. The changed parameters were: concentration of Cu (II) (1.5 mM or 15 mM), aqueous-acetonic and aqueous-methanolic solutions (0 – 80 %) as well as pH range 3 – 8.

Each time, 20 µl of buffer, 20 µl of copper sulphate solution, and 20 µl of pigment were sequentially introduced into the wells of the microplate. Appropriate volumes of water and organic solvent were added to obtain the final concentrations.

pH ranged from 3 to 8, at 0.5 pH-unit step samples were allotted to 1 – 12 columns of 96-well microplates. In rows A to H, concentrations of organic solvents differed from 0 to 80 % (v/v). In the experiment, acetate (3 – 5.5) and phosphate (5.5 – 8) buffers were used. 24-hour spectrophotometric analysis was carried out at 25°C in each case. The calculated optical path-length for a volume of 200 µl in a microplate well was 0.53 cm. The reaction was stopped by addition of 20 µl of EDTA.

3. Results and discussion

The absorption maximum of 17-decarboxy-betanin (17-dBt) is located in its Visspectrat 505 nm. Comparing the intensity of this band in spectra measured during the experiments (Fig. 2 – 3), a decrease of the maximum during 24 h clearly suggests decomposition of this compound under the influence of Cu (II). The longer the reaction time, the more pigment molecules degrade. But the pH value and the concentration of organic solvents are also important factors in this study.

In most cases, the pigment is more stable at pH 6 – 7. More acidified and more basic solutions accelerate the degradation of 17-dBt and cause a hypsochromic shift of the main band in the spectra (Fig 2-4).

Moreover, in many spectra, new absorption bands are appear in the range of 370 – 480 nm. Based on a previous study, formation of new compounds which can be
Fig. 2. UV-Vis spectra of 17-decarboxy-betanin after 2 h incubation in 45 – 80 % acetonic-aqueous and methanolic-aqueous solutions with 1,5 mM Cu (II) cation.

Fig. 3. UV-Vis spectra of 17-decarboxy-betanin after 10 h incubation in 45 – 80 % acetonic-aqueous and methanolic-aqueous solutions with 1,5 mM Cu (II) cation.
products of 17-decarboxy-betanin decomposition is suggested (Wybraniec, 2013) as a result of possible oxidation leading to the formation of dehydrogenated as well as di- and tridecarboxy-derivatives.

Comparing the influence of the concentration of organic solvents, the decomposition of the tested pigment is more rapid in acetonic-aqueous solutions, because after 10 h incubation with acetone addition most of the 17-dBt main absorption bands had lower intensity than the respective bands in metanolic solutions (Fig. 2 – 3). There are no significant changes between the samples containing 45 % organic solution concentrations. In general, the results show lower tendency for forming new bands in metanolic-aqueous solutions (Fig. 3 – 4). In addition, it can be noticed that after 10 and 24 h of 60 % as well as 80 % methanolic-aqueous solutions there are not distinctive changes between the spectra.

However, in both cases, the influence of concentration of the organic solvent is crucial. Along with an increase in the organic solvent concentration, the tendency for pigment decomposition increases. It is shown, for instance, in spectra for 80 % methanolic-aqueous solutions, where the main band of 17-dBt disappears (Fig. 3 – 4).

The increase of Cu (II) cations concentration results in an acceleration of 17-decarboxy-betanin decomposition, which is proved by the formation of new bands in the UV-Vis spectra just after 2 h in the case of 15 mM Cu (II), even at low amounts of the organic solvent. This data are shown in Fig. 4b in comparison to corresponding spectra of 10 times lower Cu (II) concentration, where no other maxima appear (Fig. 4a).
The evident negative impact of higher (15 mM) concentration of Cu (II) is proved by spectra collected after 24 h (Fig. 5). Surprisingly, the results present a characteristic tendency for the formation of degradation products in samples at pH 3 – 5. 17-decarboxy-betanin is quite stable at pH 6 – 8 even at 15 mM Cu (II) irrespective of the type of the organic-aqueous solution.
4. Conclusions

The concentration of copper is a deciding factor for the decomposition rate of 17-decarboxy-betanin. Moreover, the aqueous-acetonic solutions turned out to be more detrimental than the methanolic solutions. The concentration of organic solvents is also significant and in each case, its increase results in an acceleration of the pigment degradation. This research has to be completed by LC-MS analyses data to collect more information about the degradation products.

References