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Color stabilization in aqueous solution of Beetroot Red

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Abstract. Aqueous solutions of Beetroot Red fade fairly quickly, when kept in daylight, due to betanin hydrolysis. Aqueous solution produced out of Beta vulgaris chips is an unstable disperse solution. Protein coagulation results in turbidity and residuum in the solution under storage conditions. Proteins and the colorant form hydrogen bonds. Under coagulation of these bonds, the colorant is released and colorant hydrolysis is initiated. Hindered protein settling leads to the colorant precipitate together with the protein. In order to stabilize the colorant solution, we have used colloid protection that helped the solution retain the color continuously. Guar gum was used as a high-molecular compound to stabilize the color of the solution continuously.

1. Introduction

The interest towards natural colorants is fueled by the fact that bright synthetic colorants used in the food industry and pharmaceuticals are detrimental to health. There are many colorants produced from natural raw materials, such as curcumin, carmine, anthocyanin colorants, and betalains. Many of them have a non-stable color, so the actual task is to find conditions and stabilizers that will allow them to be used for a long time. Beetroot Red is one of these colorants.

Beetroot Red contains a natural pigment that belongs to the group of betalains [1]. This pigment group has a limited distribution in nature and is well known for coloring the roots of beetroot (Beta vulgaris). After a day in the daylight, an initially clear solution of Beetroot Red begins to grow turbid, resulting in a white residuum, while after five days, the solution becomes discolored. In this form, solutions of Beetroot Red cannot be used. In an aqueous solution, the betanin molecule undergoes hydrolysis [2]. And accordingly, the colorant solution loses its spectral properties.

2. Materials and methods

This research involved beetroot chips – large thinly sliced dried strips of peeled beetroot (Beta vulgaris). This form of a natural product was used to reduce the amount of solid inclusions in the solution. The aqueous solution was infused at room temperature and filtered through a fine-pore filter. The concentration of Beetroot Red was identified as 0.007 mol/L. through the use of E-162 calibration chart by ParfaitDecor.

The change in color of the solution was controlled by the change in optical density/turbidity with the help of KFK-3M. The wavelength identified as $\lambda_{\max} = 540$ nm, which corresponds to the maximum absorption of betanin [3].



In order to achieve stabilization, we used edible guar gum, with a concentration of 0.05 g/ml. The guar gum went through preliminary preparation: it was diluted in water with constant stirring to a homogeneous solution. At high concentrations, guar gum is converted to a gel without heating.

A U-shaped vessel was used for electrophoresis. It was filled with a colorant solution, with a current strength of 2 A and a voltage of 2 V.

3. Results and discussion

Beet root is a complex multicomponent system, respectively, the solution obtained from the dry product is also a multi-component system. Despite the initially ideal clarity of the solution, it is a disperse system.

To solve the problems of colorant solutions stabilization, the solution was tested for the properties of disperse systems:

1. one of the signature properties of these systems is the Tyndall cone, which was observed in the solution under study;
2. the colorant solution was subjected to electrophoresis, which resulted in solution separation into two parts. That indicates the presence of a molecular charge.

The sediment formed after coagulation was tested with qualitative reactions to proteins, biuret and ninhydrin, both reactions gave a positive result. Therefore, it can be affirmed that it's the proteins that fall into the residuum, when a solution of Beetroot Red is stored at room temperature. Even after thorough filtration, the colorant solution obtained from the beetroot chips, contains fiber particles that provoke coagulation of the proteins, when precipitating. It is noted that the more solids there are in the solution, the faster the colorant degrades in it. Probably, the protein and betanin molecules form hydrogen bonds. A breach in those bonds results in a loss of stability of the colorant solution, which leads to accelerated hydrolysis along the aldimine bond [4].

To determine the starting point of the hydrolysis, optical density was measured during the first three hours after the solution was prepared. Figure 1 shows that changes in the solution structure appeared immediately after its preparation, which means hydrogen bonds between the protein and the colorant weaken due to formation of protein conglomerates. During this time, the solution itself remains clear. Only when a certain concentration of coagulant is reached, the perceptible coagulation process begins, leading to solution turbidity and visible to the human eye.

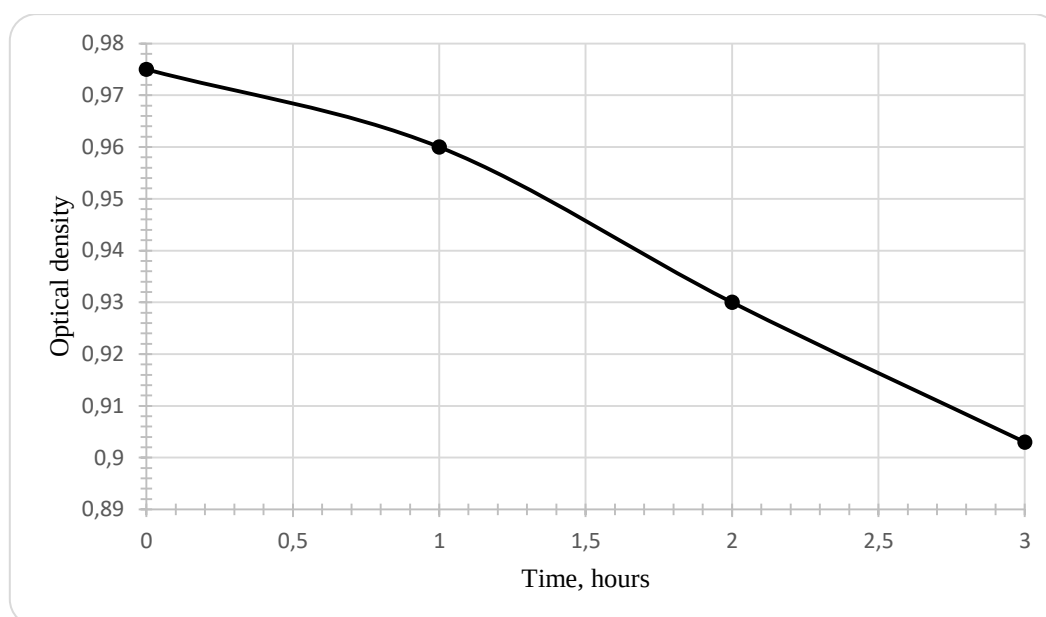


Figure 1. Dependence of the initial change in the optical density of Beetroot Red solution on time.

Colloidal protection with a high molecular weight compound (HMWC) was used to stabilize the color of the aqueous solution of Beetroot Red. For this purpose, we used guar gum, a polysaccharide that functions as a thickener in the food industry (figure 2). An increase in the solution viscosity is one of the reasons for its longer storage time.

Its solutions form structured fluids. The gum molecule hydrates through a large number of hydrophilic groups (their presence is indicative for polysaccharides) and stretches into filamentous macromolecules, forming various bonds with protein and colorant molecules, which prevents protein coagulation and, accordingly, betanin decomposition. Since protein and colorant molecules are interconnected, the delay in protein coagulation also leads to an increment in preservation time of the colorant molecule. It should be noted that addition of guar gum results in color preservation, even when a certain amount of protein precipitates.

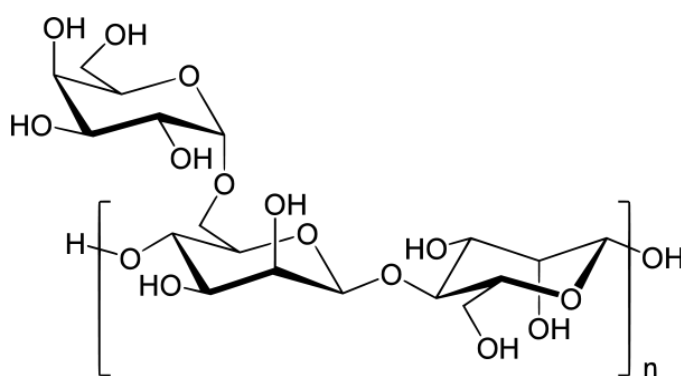


Figure 2. Guar gum.

The protein, precipitated about a day after the preparation of the solution, is white. With forced precipitation by lead acetate, the precipitate turns red violet. It is known that a complex of proteins containing lead salts is white, therefore, a colorant is present in the residuum. This confirms bonds between betanin and protein exist. Thus, the protein initiates the colorant hydrolysis, forming large conglomerates, hydrogen bonds between the protein and the colorant are destroyed, which facilitates the attack of water molecules on the massive betanin molecule.

The addition of a dismal amount of guar gum stabilized the colorant solution for a long time. The solution retained its color for three months. In comparison, the usual solution became discolored completely in 120 hours.

Figure 3 shows optical density/turbidity as a function of the solution's exposure to light. Solutions with different guar gum content were used for the experiment. The bend in curve 1 of the colorant-free solution containing no gum is associated with protein coagulation and significant turbidity. It can be seen that after adding 0.1 g/L of guar gum, curve 4 preserves the initial solution's geometry, which indicates a sufficiently strong mutual influence of protein and colorant and the lack of HMWC. At the concentration of 0.2 mol/L (curve 3), the effect is visible, the curve straightens out, i.e. there is no sharp coagulation. The optimum content of guar gum (curve 2) is 1 g/L – the stabilization effect.

To prevent fermentation of the colorant solution, ascorbic acid has been added, which has antioxidant and antibacterial properties. After the acid was added, the following effects were observed: transparency maintained, no fermentation, turbidity or sediment. A different pH of the solution changed the charge of the protein: since the proteins are ampholytes, they precipitate when lose their charge. Therefore, addition of the acid makes the proteins charged and water soluble. But the acidic media adversely affects the gum, since in acid solutions it is hydrolyzed to monosaccharides.

Therefore, figure 4 shows a decrease in the color intensity due to addition of ascorbic acid. But the solution remains sufficiently colored after 250 hours. This experiment showed that a change in pH

towards acidic values favorably affected stability. Future research will focus on the search for efficient acid additives that do not destroy guar gum and inhibit both coagulation and, accordingly, hydrolysis.

In the colorant solutions with and without guar gum, the charges of the sol were determined by a capillary method through the use of filter paper.

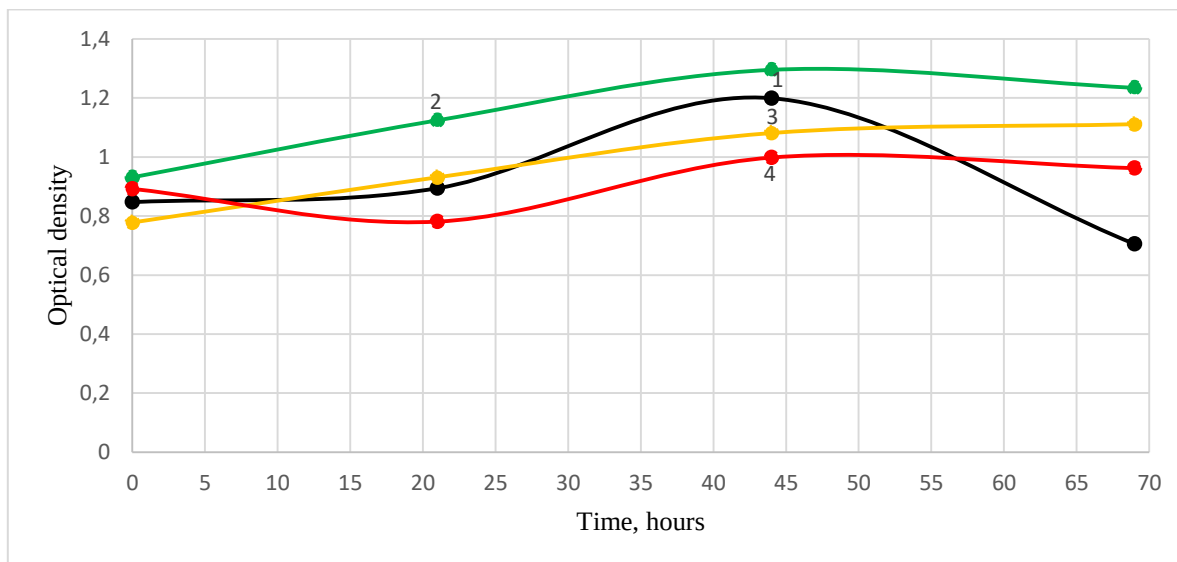


Figure 3. Dependence of Beetroot Red aqueous solutions degradation with and without guar gum on time. 1 – concentration of Beetroot Red – 0.007 mol/L; 2 – concentration of Beetroot Red – 0.007 mol/L and concentration of guar gum – 1 g/L; 3 – concentration of Beetroot Red – 0.007 mol/L and concentrations of guar gum – 0.2 g/L; 4 – concentration of Beetroot Red – 0.007 mol/L and concentration of guar gum 0.1 g /L.

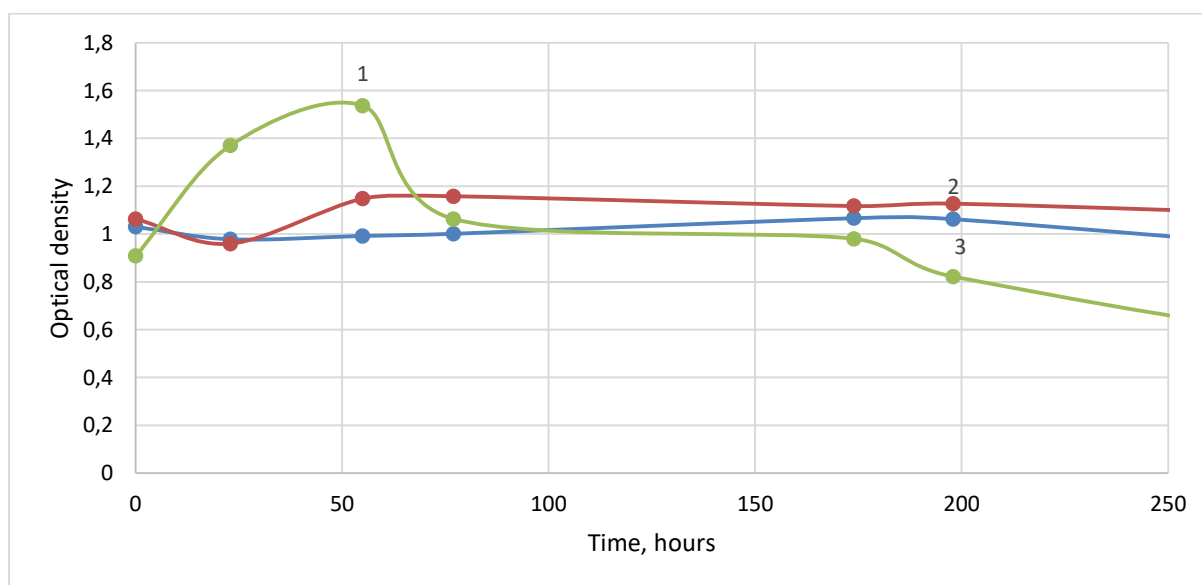


Figure 4. Comparison of the degradation time of the Beetroot Red colorant in aqueous solutions with various additives. 1 – concentration: Beetroot Red 0.007 mol/L; 2 – concentration: Beetroot Red 0.007 mol/L, guar gum 1 g/L; 3 – concentration: Beetroot Red 0.007 mol/L, guar gum 1 g/L and ascorbic acid 0.057 mol/L.

Figure 5 shows that both regular solutions and the ones containing guar gum and ascorbic acid have the same charge. Consequently, the additives do not affect the sol charge, and the interaction between guar gum and Beetroot Red is not based on electrostatic attraction.

The following conclusions can be made based on this research:

1. It has been established that an aqueous solution of Beetroot Red, obtained from dry beet chips, is an unstable disperse system;
2. Protein coagulation initiates the hydrolysis of the Beetroot Red colorant;
3. Adding small concentrations of guar gum (1 g/L) to the solution stabilizes the color of the solution of the Beetroot Red colorant continuously, due to solution structuring and delay of the hydrolysis;
4. Adding of 1% ascorbic acid allows one to maintain the solution transparency with a satisfactory color retention, as well as to suppress fermentation.

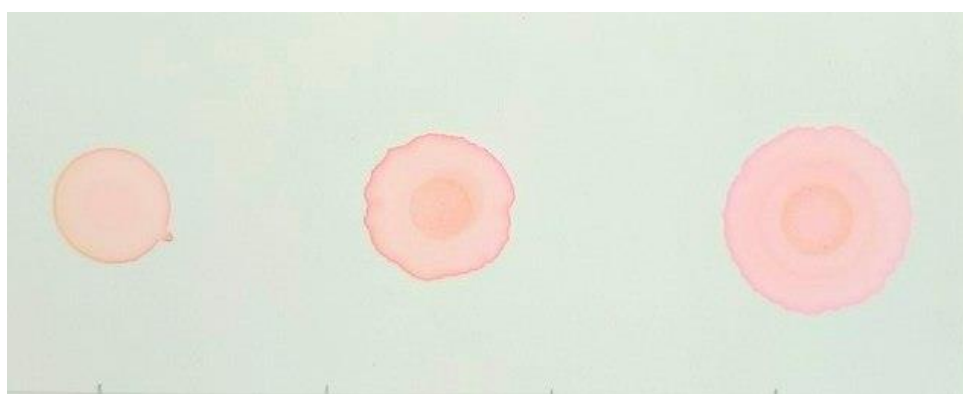


Figure 5. Drops of Beetroot Red solutions on filter paper. 1 – solution without additives; 2 – solution containing guar gum; 3 – solution containing a mixture of guar gum and ascorbic acid.

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