Evaluation of Melanoidin Contribution to Colour Characteristics of Brewing Products

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Abstract. The article focuses on the issue of melanoidin quantification and its influence on the color characteristics of brewing products. It is shown that the reaction conditions (100±2)°C, the processing time of 2 hours and a certain list of sugars and amino acids typical for brewing products are adequate to obtain linear dependencies for estimating the beer melanoidin content at 300 and 420 nm. The data on the total content of melanoidins in light beer in the range of 3.83-51.4 mg/dm3, and in dark beer – 6.82-145.94 mg/dm3 at the specified wavelengths are given. The authors found that the dependence between the color and the total beer melanoidin content is characterized by a correlation coefficient R = 0.624, which indicates the influence of other organic compounds of beer on the color intensity.

1 Introduction

The identification of beer by colour is of great importance from the point of view of the formation of consumer characteristics of this group of alcoholic beverages, since the appearance of products affects demand the most. There are standardized methods for determining colour in the industry, which are not without drawbacks in light of recent studies. It is noted that the formation of colour in beer occurs throughout the entire production technology and is characterized by the presence of melanoidins, phenolic and other compounds [1]. Due to the complexity of describing the colour of beer pigments associated with their extraction and quantification, the relevant task is to study the spectral characteristics of melanoidin solutions, with the help of which it will be possible to quantify the content of Maillard reaction products in beer. The purpose of this research was to evaluate the effect of melanoidins on the colour characteristics of beer based on the study of spectral characteristics of model melanoidin solutions.

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2 Materials and methods

Melanoidins obtained during 2-hour processing at a temperature of (100±2)°C were used as the model solutions based on mono- and disaccharides (xylose, fructose, glucose, maltose) and amino acids (lysine and glycine).

9 samples of light and dark filtered pasteurized beer purchased in the retail chain of Moscow were used in the research. The samples were stored and sealed at a temperature of (10±2)°C during the research. Melanoidins from beer samples were extracted according to [2].

Generally accepted methods were used in the research: beer colour determination – according to [1], optical density determination was carried out on a spectrophotometer MS 122 (Russia). Statistical data were processed by the Statistics program (Microsoft Corporation, Redmond, WA, USA, 2006). The experiments were carried out in 5-6 replicates with a confidence probability of p≥95%.

3 Results and discussion

To solve the tasks set, a list of amino acids (lysine and glycine) was chosen taking into account the highest rate of Maillard reaction in the beer colloid system [3], and mono- and disaccharides were chosen taking into account their presence in the beer composition [4].

Figure 1 graphically shows the change in the optical density of melanoidin solutions depending on the wavelength.

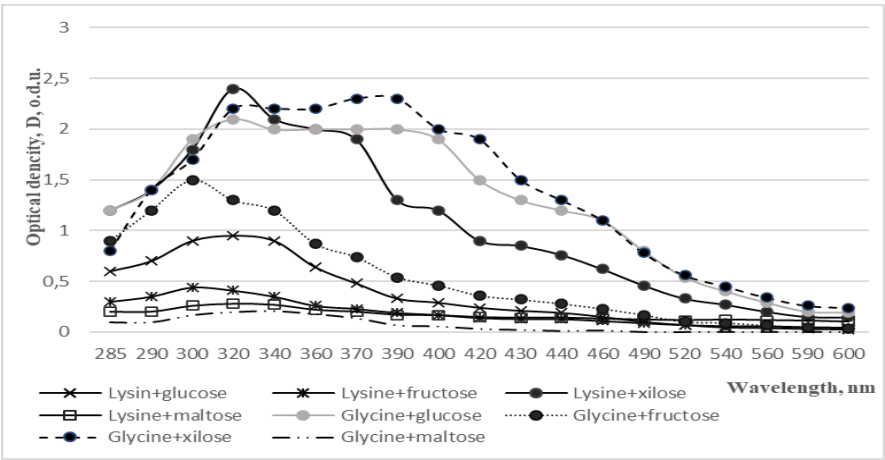

Fig. 1. Dependence of the optical density of the melanoidin solution on the wavelength.

The data of graph 1 clearly show that the colour intensity of melanoidin model solutions depends on the nature and structure of both amino acids and sugars. In the formation of melanoidins from glycine, lysine and glucose, the nature of the amino acid has a determining value: glycine with glucose gives more coloured compounds than there are with lysine. In the mutual thermal reaction between fructose, glycine and lysine, the nature of the amino acid also plays an important role: glycine with fructose gives more coloured solutions compared to lysine. In the reaction between these amino acids and xylose, a reducing substance is of crucial importance, since more coloured compounds are formed both with glycine and lysine, with maximum colour intensity compared to all other solutions. In the reaction of amino acids with maltose–disaccharide – the optical density of

melanoidin solutions is the lowest, that is, the reaction is determined by the nature of the disaccharide.

There are recent studies that claim that the type of amino acid is responsible for the molecular characteristics of the melanoidin formed, and the type of the reducing compound determines the rate of melanoidin formation reaction [5].

It should be noted the longer duration of the authors' experiment (10 hours) and the limited list of sugars as differences. However, the conclusions of the above and our research partially coincide, the discrepancies are related only to the behaviour of the reagents for a different amount of time.

The studies [6] have made it possible to establish effective light absorption by the melanoidin solutions in the range of 280–600 nm, depending on the molecular weight of melanoidin: low-molecular pyrazine components of melanoidins are absorbed in 280-300 nm, and coloured products of the 5-hydroxymethylfurfural type with high molecular weights – at 420 nm [7].

This fact is confirmed by the nature of changes in the optical density of melanoidin solutions, the most coloured of which (with the participation of xylose and lysine, xylose and glycine, as well as glucose and glycine) had maxima both in the range of 300 ± 20 nm and 420 ± 20 nm. The researchers also call pigments having light absorption in the range of 400 nm di-amino-pyrrolones, which are formed due to xylose compared to other sugars [8].

As part of the research, it was decided to measure the optical density of melanoidins in the wavelength range of 280-320 nm and 420 nm in order to fully characterize the melanoidins formed in the model experiment.

Based on the data in Figure 1, calibration graphs of the change in the optical density of melanoidin solutions from the concentration at the corresponding wavelengths were constructed. They are presented in Figure 2. The data in Figure 1 were evaluated using the Statistics program.

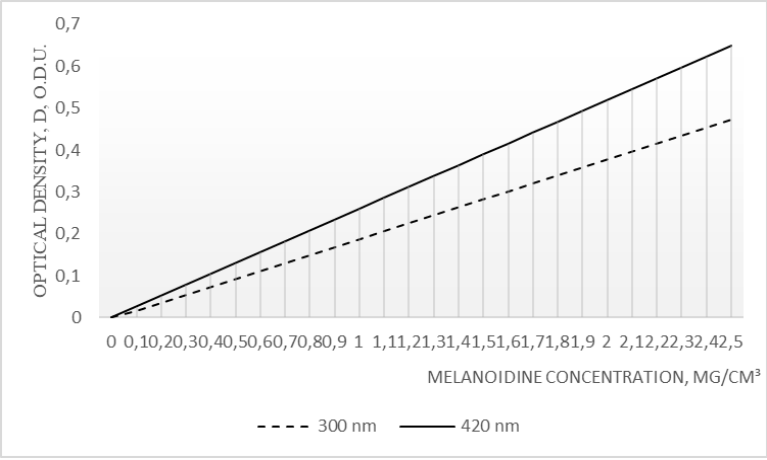

Fig. 2. Calibration graph of the dependence of the melanoidin solution optical density (D, o.d.u.) on its concentration in solution (mg/cm^3) at a wavelength of 300 and 420 nm.

Mathematical analysis has shown that the correlation between the change in the concentration of melanoidins and the optical density of solutions at wavelengths of 300 and 420 nm is linear, characterized by a correlation coefficient of 1.0 for both cases, which indicates a strong correlation. At the same time, the regression variance coefficient has 99.9% accuracy in both cases, which characterizes the calibration graphs as reliable.

The extracted beer melanoidins were quantitatively characterized using calibration lines (Figure 2) and compared with the colour characteristics of the initial beer samples, the results are presented in Table 1.

Table 1. The melanoidine content in beer's samples.

Beer's colour, °EBC	**The beer's melanoidin content, mg/L, depending on light absorption wavelength**		
	300 nm	**420 nm**	**summ**
light			
4.75±0.14	3.82±0.19	0.94±0.05	4.76
5.0±0.15	3.09±0.19	5.68±0.28	8.77
9.75±0.29	2.84±0.14	0.99±0.05	3.83
17.0±0.50	39.27±1.95	12.13±0.60	51.40
dark			
19.75±0.59	7.22±0.35	2.26±0.11	9.48
21.3±0.64	78.67±3.95	21.72±1.20	100.39
65.0±1.95	24.37±1.20	2.01±0.10	26.38
75.0±2.25	105.33±5.25	40.61±2.00	145.94
97.5±2.93	5.32±0.25	1.50±0.08	6.82

According to Table 1, a correlation was calculated between the colour of beer samples (Y) expressed in °EBC and the content of melanoidins measured at 300 nm (X_1), at 420 nm (X_2) and their sum (X_3). The data are presented in Table 2.

Table 2. Correlation coefficients between colour variables and melanoidin content in the beer samples.

-	**Colour, ° EBC**	**Correlation coefficients estimating the dependence between the melanoidin content, mg/L, measured at wavelength**					
		paire			partial		
		300 nm	420 nm	summ			
	y	x_1	x_2	x_3	x_1x_2	x_1x_3	x_2x_3
y	1	0.29	0.27	0.29	0.96	0.99	0.98
x_1	0.29	1	0.96	0.99	-	-	1
x_2	0.27	0.96	1	0.98	-	1	-
x_3	0.29	0.99	0.98	1	-1	-	-

The statistical calculation estimating the influence of melanoidin fractions on the beer colour, the results of which are shown in Table 2, shows that with a multiple correlation coefficient R = 0.83, only the sum of fractions strongly affects the level of beer colour. Thus, it is confirmed that when calculating the number of melanoidins and the associated colour index, the sum of Maillard reaction products, the light absorption of which was measured at 300 nm and 420 nm, should be taken into account.

When calculating the regression equation between the colour and the total content of melanoidins, the correlation coefficient R = 0.624 was calculated, which on the Chaddock scale indicates a noticeable connection between the parameters under consideration. However, this is not enough to compile an equation of the connection between colour and melanoidin content and to compile a correct regression equation, it is necessary to take into account other parameters: catechins, protein compounds, non-starch polysaccharides, the influence of which on the beer colour characteristics is confirmed by other studies [9-11].

4 Conclusions

The conducted studies allowed us to conclude that the beer colour characteristics are influenced by melanoidin fractions having absorption maxima at 300 and 420 nm, but they are not the only compounds responsible for the formation of beer colour. The intensity of the coloured complex based on amino acid and mono- or disaccharide depends on the nature of the reacting compounds.

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