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### RESEARCH ARTICLE

#### EXPANDED PERLITE MELANOID PIGMENT FORMATION WITH PARTICIPATION OF AMINO BENZOIC ACID

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#### Abstract

In a determinate sense, Maillard reaction is identified with generation of brown-colored compounds or melanoidins in the reaction environment. Despite this fact, the stage of colored compound formation is episodically studied only [7]. It is established that coloring is related to formation of highly-molecular compounds (> 12000 Dalton) or melanoidins. As a rule, this mass contains heterogenic mixture of melanoidins, and each of them has the different absorption maximum. The intensity of color of our compound is predetermined by high concentration of these compounds, which have intense absorption in the ultraviolet region. Using the similar techniques, we have studied the distribution of melanoid pigment ( $\lambda = 470$  nm) among low-molecular (<3500 Dalton) and highly-molecular fractions, in reaction mixture obtained via interaction of m-aminobenzoic acid and D-glucose. Results are given in the first figure. Components, which didn't enter into reaction have been determined during reaction, in samples taken from reaction mixture in the definite intervals of time. Reaction mixture has been divided on paper or in thin layer. index terms—Maillard reaction, m-aminobenzoic acid, p-aminobenzoic acid, pH – metre, melanoid pigment.

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#### Introduction:-

In a determinate sense, Maillard reaction is identified with generation of brown-colored compounds or melanoidins in the reaction environment. Despite this fact, the stage of colored compound formation is episodically studied only [7]. It is established that coloring is related to formation of highly-molecular compounds (> 12000 Dalton) or melanoidins. As a rule, this mass contains heterogenic mixture of melanoidins, and each of them has the different absorption maximum. The intensity of color of our compound is predetermined by high concentration of these compounds, which have intense absorption in the ultraviolet region. Using the similar techniques, we have studied the distribution of melanoid pigment ( $\lambda = 470$  nm) among low-molecular (<3500 Dalton) and highly-molecular fractions, in reaction mixture obtained via interaction of m-aminobenzoic acid and D-glucose. Results are given in the first figure. Components, which didn't enter into reaction have been determined during reaction, in samples taken from reaction mixture in the definite intervals of time. Reaction mixture has been divided on paper or in thin layer.

Series of studies points at the fact that a melanoid complex generated resulting from interaction between proteinic amino acids and sugars, consists not only of highly-molecular compounds; significant colored part ( $\lambda = 470$  nm) of this compound is relatively low-molecular one. For instance, as the study of products obtained resulting from

glucose/glycine mixture (pH= 5,5; at 55°C) showed, a portion of relatively highly-molecular fraction of colored products equals roughly 10%, while the rest falls at low-molecular compounds (<3500 Dalton) [7, 4, 5]. A similar study has been carried out for the systems containing glucose/glycine and glucose/alanine in the phosphate buffer at pH = 7 and 95°C temperature during 4-hour heating. It turned out that under these conditions, only a negligible quantity falls at the colored highly-molecular fraction (>3000 Dalton), while a coloration is mainly caused by low-molecular fraction [2]. Martins [5] has studied the share of highly-molecular fraction (>3500 Dalton) in the melanoid complex generated at different temperatures and different pH in the glucose/glycine model system. As is turned out, among the compounds, which are responsible for absorption at 420 nm, the share of highly-molecular fraction comprises no more that 20%.

Using the similar techniques, we have studied the distribution of melanoid pigment ( $\lambda = 470$  nm) among low-molecular (<3500 Dalton) and highly-molecular fractions, in reaction mixture obtained via interaction of m-aminobenzoic acid and D-glucose. Results are given in the first figure.

### **Objects and Methods:-**

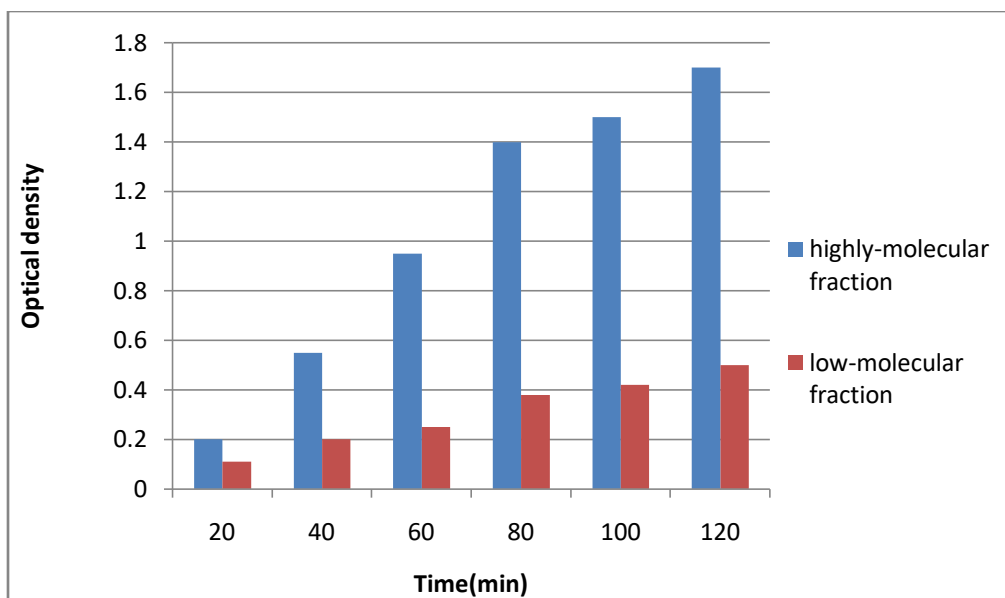
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### **Experimental Part**

We have studied melanoid pigment ( $\lambda = 470$  nm) distribution between low-molecular (<3500 Dalton) and highly-molecular (>3500 Dalton) fractions of melanoidin product generated via interaction of m-aminobenzoic acid and D-glucose. Reaction conditions: phosphate buffer, pH 7.0; 0,1M solutions, molar ration – m-aminobenzoic acid / D-glucose = 1:1, temperature 100 °C, duration 20, 40, 60, 80, 100, 120 minutes. Dialysis conditions: dialysis membranes [SPECTRA/POR]prepared from regenerated cellulose, which are able to hold the compounds with molecular mass > 3500 Dalton; dialysis against distilled water during 72 hours. Distilled water has been changed on a regular basis once in 12 hours, dialysis temperature was 15-18°C. Melanoidin has been quantitatively determined at  $\lambda = 470$  nm. Reactions between aldoses and aminobenzoic acids have been conducted in phosphate buffer medium, with pH values – 5,0; 7,0; 9,2; at temperature 100°C, concentration of reacting components has been 0,1 mole/l. Right after expiration of reaction time, the reaction mixture has been rapidly cooled down to room temperature. Melanoidin reaction has been controlled according to generated melanoid pigment extinction ( $\lambda = 470$  nm), on one side, and according to quantity of amine and aldose didn't enter into reaction, on the other. Components, which didn't enter into reaction have been determined during reaction, in samples taken from reaction mixture in the definite intervals of time. Reaction mixture has been divided on paper or in thin layer.

### **Results and Discussion:-**

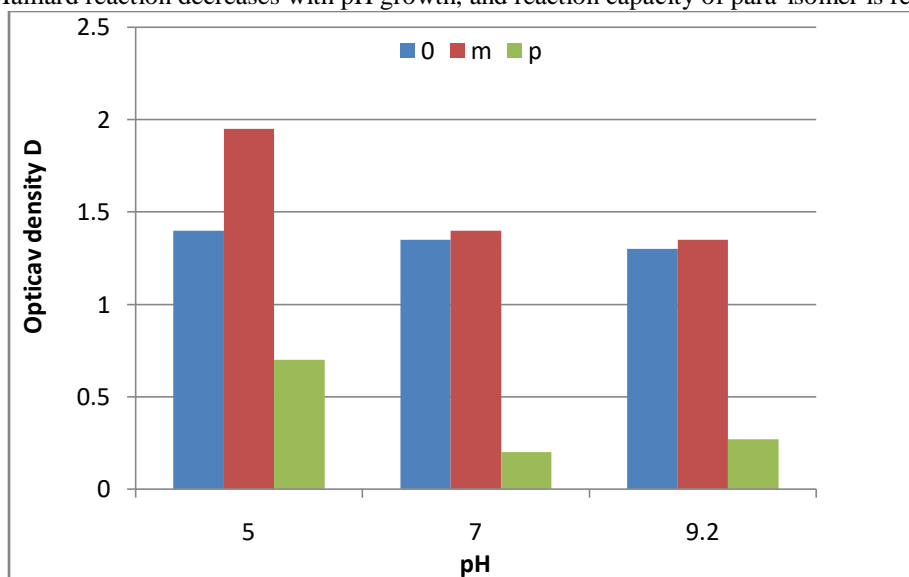
Using the similar techniques, we have studied the distribution of melanoid pigment ( $\lambda = 470$  nm) among low-molecular (<3500 Dalton) and highly-molecular fractions, in reaction mixture obtained via interaction of m-aminobenzoic acid and D-glucose. Results are given in the first figure.



**Fig. 1:-** Melanoïd pigment distribution ( $\lambda = 470$  nm) between low-molecular (<3500 Dalton) and highly-molecular (>3500 Dalton) fractions of the melanoïd product obtained via interaction of m-aminobenzoic acid and D-glucose.

As is seen from Fig. 1, the content of highly-molecular (>3500 Dalton) fraction in melanoïdin prepared via interaction of aminobenzoic acid and D-glucose is substantially higher (22-30%) than in the above-mentioned systems containing glucose/glycine and glucose/alanine. Here one has to mention the fact that in contradistinction from glycine and  $\alpha$ -alanine, which react with relatively lower intensity with glucose while forming melanoïdin, aminobenzoic acids in this regard are characterized by much higher activity. This is particularly so in case of m-aminobenzoic acid, which reactivity (reactive capacity) towards aldoses is especially high and, at the same time, in most case, it generates melanoïdin pigment insoluble in water and buffer system. Its reaction capacity is particularly high in acidic medium, that will be discussed in detail below.

As opposed to protein amino acids, which take more active part in Maillard reaction in case of alkaline medium, isomeric aminobenzoic acids react more vigorously in acid environment. The ability of aminobenzoic acids to participate in Maillard reaction decreases with pH growth, and reaction capacity of para-isomer is relatively low

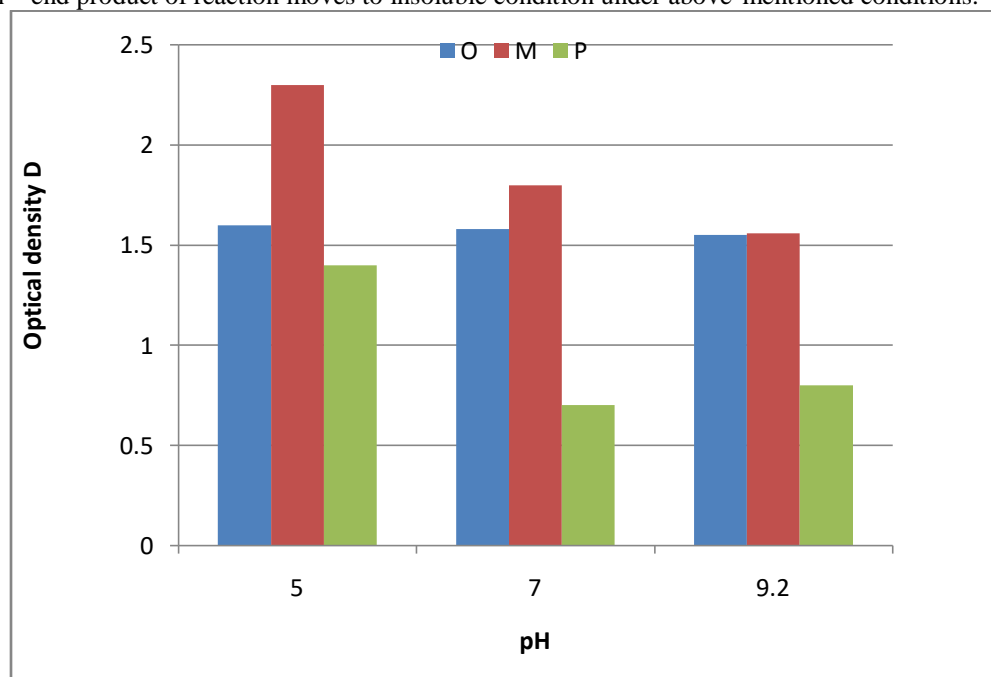


**Fig. 2:-** Melanoïdin formation via o-, m- and p-aminobenzoic acid interaction with D-glucose in acidic, neutral and alkaline mediums (reaction conditions: phosphate buffer, 0,1 M solutions, molar ratio – amino acid/glucose = 1:1, temperature 100 °C, duration 2 h,  $\lambda = 470$  nm)

As is seen from obtained data, interaction of aminobenzoic acids and D-glucose is subject to some regularities. Quantity of amino acids entering the reaction increases with temperature rise. At that, in acid, neutral and alkaline environments m-aminobenzoic acid takes far more active part in reaction, than p-aminobenzoic acid, while o-isomer takes intermediate position in this regard. Probably it can be explained by different basicity of isomeric aminobenzoic acids (pKa value of o-, m- and p-aminobenzoic acids equals 2,11; 3,12; 2,41, respectively [3]).

The fact that aminobenzoic acids ability to participate in Maillard reaction decreases with growth of reaction medium pH, is a common pattern and is confirmed in case of each aldose. For example, this picture is observed during reaction between aminobenzoic acid and D-galactose (Fig. 3).

Compared to aldohexoses, aldopentoses take more active part in melanoidin generation process. For example, aminobenzoic acids so actively participate in reactions with aldopentoses (xylose, arabinose), that the major part of melanoidin – end product of reaction moves to insoluble condition under above-mentioned conditions.



**Fig.3:-** Melanoidin formation via o-, m- and p-aminobenzoic acid interaction with D-galactose in acidic, neutral and alkaline mediums (reaction conditions: phosphate buffer, 0,1 M solutions, molar ratio – amino acid/galactose = 1:1, temperature 100 °C, duration 2 h,  $\lambda = 470$  nm)

First stage of Maillard, or sugar-amine, reaction is represented by amine N-glycosylation [1, 6]. N-glycosidic bond is labile and easily undergoes hydrolyzation in weakly acid and alkaline, or neutral medium, but the second main path of its transformation under the same conditions is Amadori rearrangement, which leads to formation of melanoidin products [8]. The subsequent transformation of Amadori rearrangement product depends on reaction medium pH; in neutral and acid environments it mainly experiences 1,2-enolization, as result of which furfural (from pentoses) or hydroxymethylfurfural (from hexoses) is generated, while in alkaline medium it mainly undergoes 2,3-enolization, which is followed by formation of reductones and splitting products (4-hydroxy-5-methyl-2,3-dihydrofuran-3-on, acetol, pyruvate aldehyde etc.). All these products are extremely reactive and intensely participate in melanoidins formation.

### Conclusions:-

Aldoses and melanoidins have been isolated from aminobenzoic acids in the system of dioxane-benzol taken in 1:1 or 1:4 ratio; in its turn, aldoses and melanoidins have been separated from each other in the ethanol-water (9:1) system. P-dimethylaminobenzaldehyde has been used as a developer for aminobenzoic acids, while aniline phthalate or solution of silver nitrate in ammonia hydroxide has been applied for aldoses.

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