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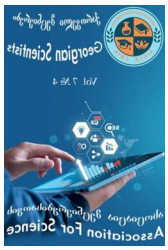

Fractional composition of melanoidin pigment

Tserodze Nana[1], Tatiashvili Leila[2], Tsiskarishvili Rusudan[3], Dolidze Liparit[4], Bolkvadze Nona[5], Karkashadze Nino[6], Uridia Rusudan[7]

[1]PhD in Chemistry sciences, Scientist of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia. nana.tserodze@tsu.ge; [2]PhD in Chemistry sciences, Scientist of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia. leila.tatiashvili@tsu.ge; [3]PhD in Chemistry sciences, Senior Researcher of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia. rusudan.tsiskarishvili@tsu.ge; [4]PhD in Chemistry sciences, Senior Researcher of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia. [5]PhD in Chemistry sciences, Associate Professor of Georgian Technical University. Tbilisi, Georgia. nona.bolkvadze63@gmail.com [6]PhD in Chemistry sciences, Senior Researcher of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia. nino.karkashadze@tsu.ge [7]PhD in Biology sciences, Senior Researcher of Iv.Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia; Associate Professor of European University, Tbilis, Georgia; Orcid https://orcid.org/0009-0002-0685-2813 rusudan.uridia@tsu.ge, rusudan.uridia@eu.edu.ge

Abstract

Melanoidin complex formed as a result of interaction between protein amino acids and sugars consists not only of high-molecular compounds, but its important colored part is relatively low-molecular. Based on the example of interaction between glycine labeled preparations and D-glucose, we set a goal of study the regularities of glycine carbon atoms inclusion into melanoidin polymers. Carbon atoms of amino acid carboxyl group are quite actively included into melanoidin pigment composition and presumably, this occurs by means of products formed resulting from Amadori rearrangement. In this regard, interesting results have been obtained resulting from study of polymeric products of Maillard reaction going between D-glucose and 1-alanine. Results of element analysis of polymers obtained by us (>3500 Daltons) show that CN ratio, as one of the main parameters of these polymers, is smaller for products formed at pH8,0, than those formed at pH 5,8. Such regularity, when CN ratio of melanoidin polymers decreases with increase of pH, is described for products of alanine and glucose interactions. At that, resulting from autoradiographic analysis of acidic hydrolysates of the mentioned melanoidin polymers, there was no free radioactive glycine found in these hydrolysates. Thus, glycine is included

into polymer composition not in the form of united molecular chain, but as a molecule fragment. Based on these data one may conclude that roughly 65% of methylene carbon and approx. 14% of carboxyl carbon of glycine molecule are incorporated into melanoidin polymers under conditions we have studied. The melanoidin polymer obtained at 100°C temperature and pH 5,8 reacts with glycine at pH 8.0, the melanoidin polymer obtained at 80°C temperature reacts with glycine at 100°C temperature etc. The degree of glycine labeled carbon's inclusion into melanoidin is more highly influenced by pH change than by temperature rise.

Keywords: Maillard reaction, melanoidin pigment, protein amino acids, melanoidin complex, alanine, glucose.

Melanoidin complex formed as a result of interaction between protein amino acids and sugars consists not only of high-molecular compounds, but its important colored part (λ = 470 nm) is relatively low-molecular. For instance, as the study of products obtained resulting from glucose/glycine mixture heating (pH 5,5; 55°C) made clear, a share of relatively high-molecular fraction of colored products comprises approx. 10% only, while the rest falls to the share of low-molecular compounds (>3500 Daltons) [1,2]. The similar study has been conducted on the system containing glucose/glycine and glucose/alanine mixtures on the phosphate buffer, at pH = 7 and when heated for 4 hours at 95°C temperature. It turned out that under these conditions, only a minor part falls to the share of colored high-molecular fractions (> 3000 Daltons), while a coloration is mainly predetermined by low-molecular fraction [3]. Martins [1] has studied the share of high-molecular fraction (>3500 Daltons) in the melanoidin complex formed in the glucose/glycine model system at different temperatures and different pHs. As is turned out, only 20% falls to the share of high-molecular fraction among the compounds, which provide absorption at 470 nm.

Using the similar technique, we have studied distribution of melanoidin pigment (λ = 470 nm) between low-molecular (< 3500 Daltons) and high-molecular (> 3500 Daltons) fractions in the reaction medium obtained via interaction of m-aminobenzoic acid and D-glucose. Results are presented in Fig. 1. [4]

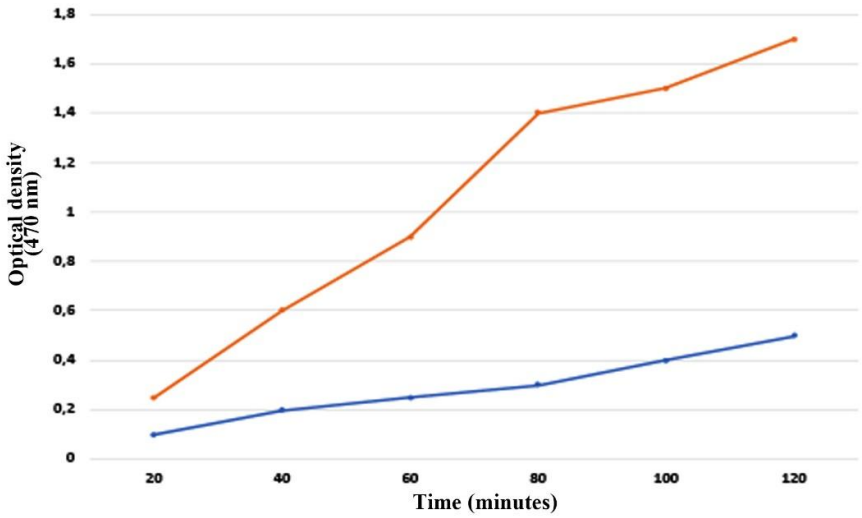

Fig. 1. Melanoidin pigment (λ = 470 nm) distribution between low-molecular (< 3500 Daltons) and high-molecular (> 3500 Daltons) fractions as a result of dialysis of reaction mixture obtained via interaction of m-aminobenzoic acid and D-glucose (reaction conditions: phosphate buffer, pH 7,0; 0,1M solutions, molar ratio: m-aminobenzoic acid / D-glucose = 1:1, temperature 100°C, duration – 20, 40, 60, 80, 100 and 120 minutes. Dialysis conditions: dialysis bags manufactured from regenerated cellulose [SPEcTRRA/R®], which retains compounds with molecular mass > 3500 Daltons; dialysis to the distilled water for 72 hours. Distilled water was changed on a regular basis once in 12 hours, dialysis temperature was 15-18°C. Quantitative determination of melanoidin was made at λ = 470 nm)

1 – low-molecular (< 3500 Daltons) fraction
2 – high-molecular (> 3500 Daltons) fraction

Based on the example of interaction between glycine labeled preparations and D-glucose, we set a goal of study the regularities of glycine carbon atoms inclusion into melanoidin polymers [5]. We have used in the experiments the glycine preparations labeled with radioactive carbon (1 ^{14}C-glycine and 2 ^{14}C-glycine), specific radioactivity of which was the same and equaled 3,0x10^{8} Becquerel/gram. Reaction between D-glucose and 1 ^{14}C-glycine or 2 ^{14}C-glycines was carried out in the phosphate buffer (pH 5,8 or 8,0), at 100°C for 5 hours. Using dialysis, melanoidin polymers (>3500 Daltons) have been purified from low-molecular admixtures. We have determined ^{14}C inclusion rate, ratio of C/N and ^{14}C/N in purified melanoidin fractions. Diagram of dialysis of melanoidin products formed at pH – 5,8 is given in Fig. 2.

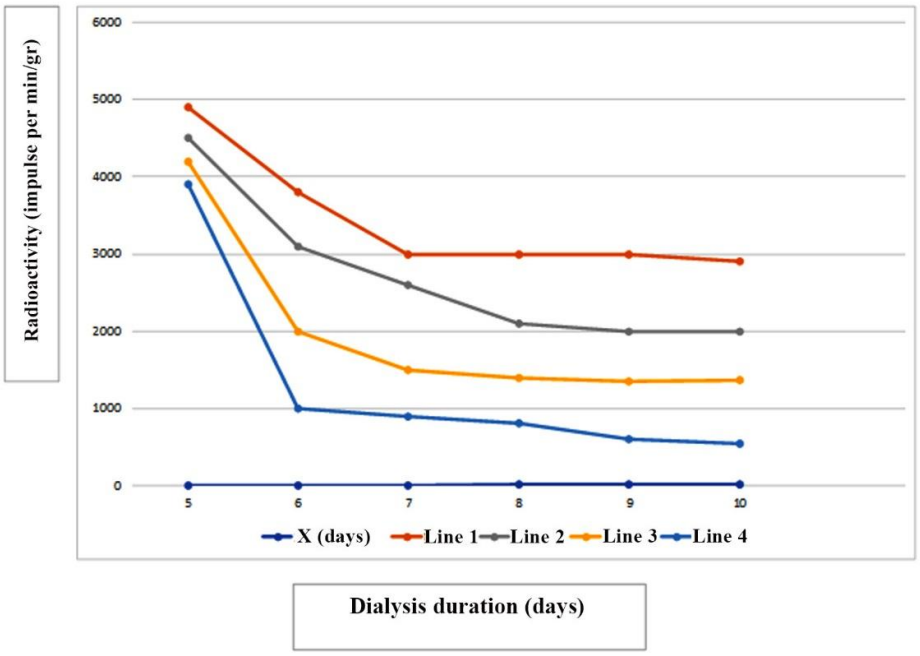

Fig. 2. Diagram of final phase of melanoidin products' dialysis; initial radioactivity of dialysis mixture is 3,37. 10^6 Becquerel/g. The product obtained on the basis of: 1 – D-glucose and 2^{14} C- glycine (pH 8.0); 2 - D-glucose and 1 ^{14}C-glycine (pH 8,0); 3 – D-glucose and 2^{14}C-glycine (pH 5,8); 4 – D-glucose and 1^{14} C glycine (pH 5,8). Dialysis conducted in the regenerated cellulose membrane (SPECTRA/POR®) 3. Membrane retains compounds with molecular mass > 3500 Daltons). Regular change of distilled water every 12 hours, 15-18°C.

Diagram of melanoidin products' dialysis shows that the rate of glycine carbon atoms' inclusion into melanoidin polymers (> 3500 Daltons) is notably higher in case of formation of these products at pH 8,0 (curves 1 and 2), than at pH 5,8 (curves 3 and 4). Such regularity is explained by the fact that a number of protonized glycine amino groups increases and the capacity to interact with glucose goes down with decrease of pH.

As we mentioned above, as a result of decay according to Strecker scheme (synthesis) H-CHO and $^{14}CO_2$ are formed along with $1^{14}C$, while H-^{14}CHO and CO_2 are generated along with 2 ^{14}C. The number of labeled carbons included into melanoidin pigment is far more in case of 2^{14}C-glycine, than of 1^{14}C-glycine (Figure 2). This points at the fact that methylene carbon of glycine is mainly incorporated into melanoidin pigment composition; presumably, such inclusion takes place by means of formaldehyde formed resulting from amino acid decay. There are multifarious ways of such inclusion [2].

Based on the λ-amino acid decay mechanism according to Strecker, one had to presume that carbon atoms of amino acid carboxyl group would not be included into melanoidin pigment composition. However, as the curve 2 shows, such inclusion occurs and very intensely at that. This is evidenced by the curves 2 and 4 (Figure 2). Thus, carbon atoms of amino acid carboxyl group are quite actively included into melanoidin pigment composition and presumably, this occurs by means of products formed resulting from

Amadori rearrangement. In this regard, interesting results have been obtained resulting from study of polymeric products of Maillard reaction going between D-glucose and 1-alanine [3]. In particular, a polymeric fraction (λ 1600 Daltons) has been obtained using labeled glucose and alanine in Maillard reaction. With the use of ^{13}C-λ MP it has been established that alanine C1 and C2 atoms are included into polymer, while glucose C1 atom is in the form of substituted methyl group. These data coincidence well with results of the curve 2 shown in Figure 1 and curves 2 and 4 demonstrated in Figure 2.

Results of element analysis of polymers obtained by us (>3500 Daltons) show that CN ratio, as one of the main parameters of these polymers, is smaller for products formed at pH8,0, than those formed at pH 5,8 (Table 1). Such regularity, when CN ratio of melanoidin polymers decreases with increase of pH, is described for products of alanine and glucose interactions [6].

Table 1

Element analysis data of dialysed melanoidin polymers (> 3500 Daltons)

Melanoidin polymers (>3500 Daltons), obtained from:	**C/N**	**^{14}C/N**
D-glucose (0,015M) and 14 C glycine (0,015M), pH 5,8; (phosphate); 100°C, 5 hours	11.5	0.7
D-glucose (0,015M) and 2 14C-glycine (0,015M), pH 5,8; (phosphate); 100°C, 5 hours	11.2	1.1
D-glucose (0,015M) and ^{14}C-glycine (0,015M), pH 8,0; (phosphate); 100°C, 5 hours	9.3	0.7
D-glucose (0,015M) and 2 14C-glycine (0,015M), pH 5,8; (phosphate); 100°C, 5 hours	9.7	1.1

^{14}CN ratio shows the degree of inclusion of nitrogen and labeled carbon of glycine molecule into melanoidin polymers (Table 1). For glycine molecules this ratio CN = 1,7, while in all polymeric products studied by us, the degree of glycine carbon atoms inclusion is lower. At that, resulting from autoradiographic analysis of acidic hydrolysates of the mentioned melanoidin polymers, there was no free radioactive glycine found in these hydrolysates. Thus, glycine is included into polymer composition not in the form of united molecular chain, but as a molecule fragment. Based on these data one may conclude that roughly 65% of methylene carbon and approx. 14% of carboxyl carbon of glycine molecule are incorporated into melanoidin polymers under conditions we have studied.

Table 2

Inclusion of 2 ^{14}C-glycine carbon atoms into composition of dyalised polymeric melanoidin (>3500 Daltons) (melanoidin : glycine = 300 mg : 0,1 mmol, glycine specific radioactivity = 3,0. 10^6 Becquerel/g)

Initial melanoidin polymer (>3500 Daltons), obtained from:	Reaction conditions	Final melanoidin polymer (>3500 Daltons)
D-glucose (0,03 M) and glycine (0,03 M) pH 5,8; (phosphate); 100°C, 5 hours	pH 8,0 (phosphate)	420 impulse per min/mg
D-glucose (0,03 M) and glycine (0,03 M) pH 5,8; (phosphate); 100°C, 5 hours	pH 5,8 (phosphate); 100°C, 5 hours	170 impulse per min/mg
D-glucose (0,03 M) and glycine (0,03 M) pH 5,8; (phosphate);100°C, 5 hours	pH 5,8 (phosphate); 100°C, 5 hours	90 impulse per min/mg
D-glucose (0,03 M) and glycine (0,03 M) pH 5,8; (phosphate); 100°C, 5 hours	pH 8,0 (phosphate); 100°C, 5 hours	130 impulse per min/mg

Table 2 data show that under the conditions different from those of melanoidin polymers receipt, these polymers may react with additional amount of glycine. For instance, the melanoidin polymer obtained at 100°C temperature and pH 5,8 reacts with glycine at pH 8.0, the melanoidin polymer obtained at 80°C temperature reacts with glycine at 100°C temperature etc. At the same time, the degree of glycine labeled carbon's inclusion into melanoidin is more highly influenced by pH change than by temperature rise (Table 2).

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