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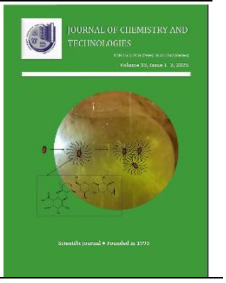

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EFFECTS OF pH AND EXTENDED HEATING ON α-diCARBONYL COMPOUNDS PRODUCED IN THE D-GLUCOSE-GLYCINE MODEL SYSTEM

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Abstract

α-Dicarbonyl compounds (α-DCs) are significant markers in the intermediate stage of the Maillard reaction, which not only provide pleasant taste and color, extend shelf life and improve functionality, but also generate a large number of potentially harmful substances that can lead to severe food safety problems and pose significant risks to human health. Therefore, food derivatives of α-DCs are under close attention in food chemistry and medicine. The study of the effects of pH and extended heating on the formation of α-dicarbonyl compounds (α-DCs) in the D-glucose-glycine model system is important for understanding methods to reduce or prevent their development in heat-processed foods. In this study the α-DCs formation were determined based on the changes in UV absorbance at λmax 270–290 nm. The results indicated that in the pH range from 1.0 to 7.0 with increasing heating time the formation of α-DCs occurred most slowly in the pH range from 4.0 to 5.0. In the pH range from 7.0 to 12.0, the slower formation of α-dicarbonyl compounds was observed in the pH range from 7.0 to 10.0. The calculated relative rates ($\upsilon = \frac{O.D.}{min.}$) of α-DCs formation in the D-glucose-glycine model system are in full agreement with the experimental data. The effect of both heating temperature and time on the formation of α-DCs in the D-glucose-glycine model system at pH 9 was investigated using Raman spectroscopy. The study of the α-DC formation patterns is to find the best and more suitable method for producing a healthy food product with good sensory characteristics.

Keywords: the Maillard reaction; Maillard reaction model system; intermediate stage; α-dicarbonyl compounds (α-DCs); intermediates.

ВПЛИВ pH І ТРИВАЛОГО НАГРІВАННЯ НА α-ДИКАРБОНІЛЬНІ СПОЛУКИ, ЩО УТВОРЮЮТЬСЯ В МОДЕЛЬНІЙ СИСТЕМІ D-ГЛЮКОЗА-ГЛІЦИН

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Анотація

α-Дикарбонільні сполуки (α-DCs) є важливими маркерами на проміжній стадії реакції Майяра, й не тільки забезпечують приємний смак і колір, подовжують термін зберігання та покращують функціональність, але й генерують велику кількість потенційно шкідливих речовин, які можуть призвести до серйозних проблем безпеки харчових продуктів і становити значні ризики для здоров'я людини. Тому харчові похідні α-DCs знаходяться під пильною увагою в харчовій хімії та медицині. Вивчення впливу pH і тривалого нагрівання на утворення α-дикарбонільних сполук (α-DCs) у модельній системі D-глюкоза–гліцин є важливим для розуміння методів зменшення або запобігання їх утворенню в харчових продуктах, підданих тепловій обробці. В цьому дослідженні утворення α-DCs визначали на основі змін УФ-поглинання за λmax 270–290 нм. Результати показали, що в діапазоні pH від 1.0 до 7.0 зі збільшенням часу нагрівання утворення α-DCs відбувалося найповільніше в діапазоні pH від 4.0 до 5.0. У діапазоні pH від 7.0 до 12.0 спостерігалося повільніше утворення дикарбонільних сполук у діапазоні pH від 7.0 до 10. Розраховані відношення швидкостей ($\upsilon = \frac{O.D.}{min.}$) утворення α-DC в модельній системі D-глюкоза–гліцин повністю узгоджуються з експериментальними даними. Вплив як температури нагрівання, так і часу на утворення α-DCs у модельній системі D-глюкоза–гліцин за pH 9 досліджували за допомогою раманівської спектроскопії. Вивчення закономірностей утворення α-DC має на меті знайти найкращий і більш відповідний метод виробництва здорового харчового продукту з хорошими сенсорними характеристиками.

Ключові слова: реакція Майяра; модельні системи реакції Майяра; проміжна стадія; інтермедіати.

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Introduction

Over a century after discovery, the Maillard reaction (L.-C. Maillard, 1912) continues to fascinate and challenge researchers around the world. The simple nucleophilic attack of an amine onto a carbonyl group gives rise to a series of parallel and subsequent reactions, occurring simultaneously, resulting into a vast array of low-mass and high-mass compounds [1; 2]. The Maillard reaction (MR) is very important for the improvement of sensory characteristics and food quality, which can promote the formation of good color, smell and taste of food, and improve the palatability of food [3–6]. The MR occurs in the human body [7] and is known as protein glycation in Health and Medical Sciences [8–10].

Hodges's description of the basic pathways of the MRs in 1953 was the first coherent and comprehensive organization of the many reactions already reported at the time to be part of the Maillard reaction. The Hodge scheme (Hodge, 1953) (divided the MR into three major stages: early stage, intermediate stage, and final stage.

As greater knowledge unfolds on a certain aspect, the need for more clarifications in another facet of this extremely complicated process becomes evident.

Among the various molecules or compounds that are formed in the intermediate stage of the reaction [11–12] are α-dicarbonyl compounds (α-DCs) [13]. α-DCs are a class of highly reactive and low-mass compounds. Although they were found only in small quantities, numerous attention has been drawn to the food field regarding their pivotal role as precursors of unique aroma, color, taste and other sensory factors [14]. As intermediate products of the Maillard reaction α - DCs can be found in almost all foods, especially those processed at high temperatures and/or have a long shelf life.

Otherwise, the existence of α-DCs may mediate the crosslinking reaction of amino acids, peptides and proteins, leading to impaired flavor, and nutritional loss of foods [15]. Hence, α-DCs are significant markers in the intermediate stage of the Maillard reaction and are closely related to the color, flavor, texture, digestibility and nutritional loss of foods [16].

α-DCs, as precursors, can not only lead to pleasant flavor and color, extended shelf life, and enhanced functionality, but can also generate the formation of potentially harmful substances such as advanced glycation end products (AGEs), what pose the potential risk to human health [17–20]. Normal levels of α-DCs have important implications for regulating human physiological metabolism, and human organisms in healthy conditions have a series of enzymes related to the function of reducing α-DCs toxicity [21].

Exogenous α-DCs are widely present in various foods, especially thermally treated and fermented foods [22]. α-DCs and their end-products are prone to accumulation in the human body. The increased intake of α-DCs with food in the body may lead to protein modification, increased oxidative stress, and chronic disease to varying degrees, such as aging, cancer, uremia and diabetic complications [23–25].

Considering that the MRs provoke both desirable and undesirable effects, the need for control is evident. The complexity of this process and the many influencing factors make this task extremely challenging.

In the last decade the strategies and control mechanisms of the highly reactive α-DCs, that can lead to severe food safety problems and pose significant risks to human health has garnered worldwide attention as a top priority. Many approaches have been proposed to control the content of α-DCs in foods, including novel processing technologies, adjusting the formula of foods, and addition of phenolic compounds, etc. [26–27]. However, effective control α-DC of daily foods remains important to help guide production strategies and result in safer and healthier foods.

Materials and Methods

Chemicals

Laboratory-made solutions of model system D-glucose- glycine sample 1 contained 0.5 mol/l of D-glucose and 0.5 mol/l of glycine. Appropriate pH values (1–7) were adjusted with HCl solution, sample 2 contained 0.5 mol/l of D-glucose and 0.5 mol/l of glycine. Appropriate pH values (7–13) were adjusted with NaOH solution.

Instruments

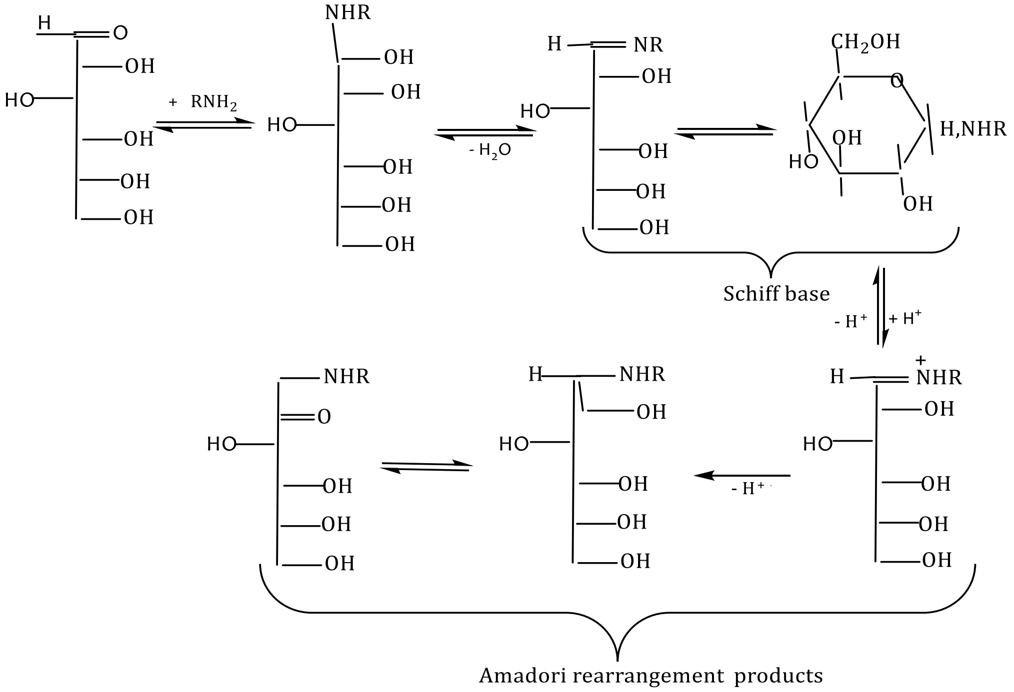
Spectrophotometer Photometry Hitachi U2810 (Hitachi High-Technologies Corporation, Japan) was used. pH values were determined by pH meter XS Instruments DHS Basic (50001202, Italy). Raman spectrometer ReactRaman (Mettler Toledo, Switzerland) was used.

Results and Discussion

«The Maillard reaction» actually consists of an extremely complex series of reactions and interactions, occurring simultaneously and sequentially, producing a vast array of low-mass and high-mass compounds.

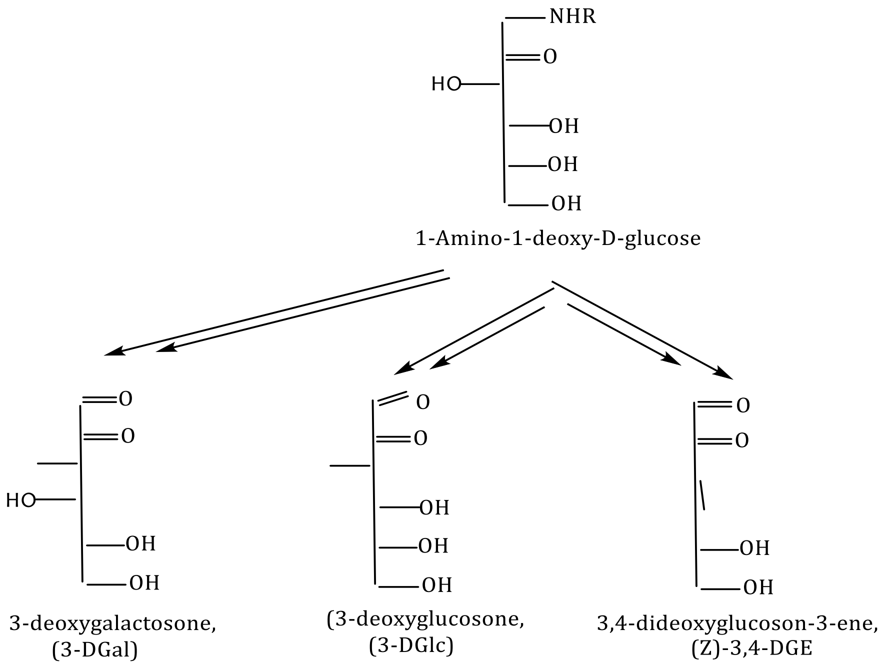
According to the Hodge-scheme (Hodge, 1953), the whole process of Maillard reaction occurs in three different stages i.e., early stage, intermediate stage, and final stage.

The initial (or early) stage consists of sugar-amine condensation and the Amadori rearrangement. The products are colorless, without absorption in the ultraviolet region



Scheme 1. Transformation in the early stage of the Maillard reaction

In intermediate stage Amadori rearrangement products (ARPs) being prone to rapid degradation to generate of α-DCs, such as d-arabino-hexo-2-ulose (d-glucosone, GLUC) as well as 1-deoxy-d-erythro-hexo-2,3-diulose (1-deoxyglucosone, 1-DGlc) and 3-deoxy-d-erythro-hexo-2-ulose (3-deoxyglucosone, 3-DGlc) [28]. The α-DCs are colorless and absorb in the carbonyl region λ_{max} 285-290 *nm* of the UV spectrum.



Scheme 2. Transformation in the intermediate stage of the Maillard reaction

Considering that α-DCs are significant markers in the intermediate stage of the Maillard reaction and provoke both desirable and undesirable effects, the need for control is evident.

The Maillard reaction is influenced by several factors such as temperature, time, pH [29]. Among these factors, the pH is believed to play an important role.

Therefore, it was logical to conduct a comparative study on the formation of α-DCs in D-glucose-glycine model system, at different pH values. The α-DCs formation at different initial pH values (1, 3, 4, 5, 7–13) was performed using UV spectroscopy methods.

In order to elucidate the effects of pH and extended heating on the α-DCs formation, D-glucose-glycine model solutions [sample 1] at the pH range between 1.0 and 7.0 were heated to 95 °C. After certain time intervals of reheating, α-DCs formed at each pH were determined based on the changes in the absorption of UV (λ_{max} 290 nm).

Analysis of changes in the intensities of carbonyl absorption suggested that heating time accelerates the α-DCs formation in the pH range between 1.0 and 7.0 but to a lesser extent in the pH range between 4.0 and 5.0 (Fig 1).

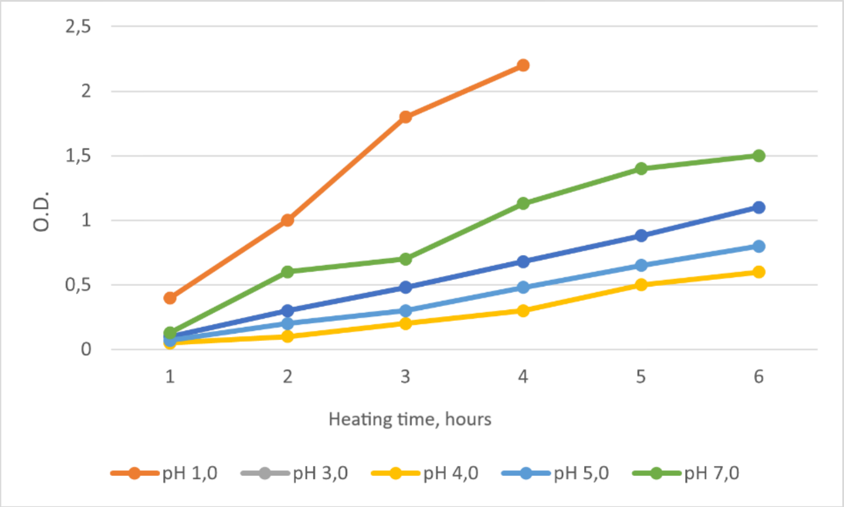


Fig. 1. Change in optical density O.D. λ_{max} 290nm of D-glucose-glycine model solutions as function of extended heating (0.5 mol/l of each compound, H_2O, 6 hours)

A comparative assessment was carried out on the pH effect (from 1.0 to 7.0) of α-DCs formation in the D-glucose-glycine model system. The solutions [sample 1] after 4 reheating hours, α-DCs formed at each pH were determined based on the changes in the absorption of UV (λ_{max} 2290 nm).

According to the influence of pH on α-DCs formation in the D-glucose-glycine model system, the pH scale (from 1.0 to 7.0) can be divided into several zones (Fig. 2).

- in the pH range between 1.0 and 4.0, the carbonyl absorption intensity rapid decreased as the pH increased

- in the pH range between 4.0 and 5.0 the carbonyl absorption intensity remains constant as the pH increased

- in the pH range between 5.0 and 7.0 the carbonyl absorption intensity increases slightly as the pH increased

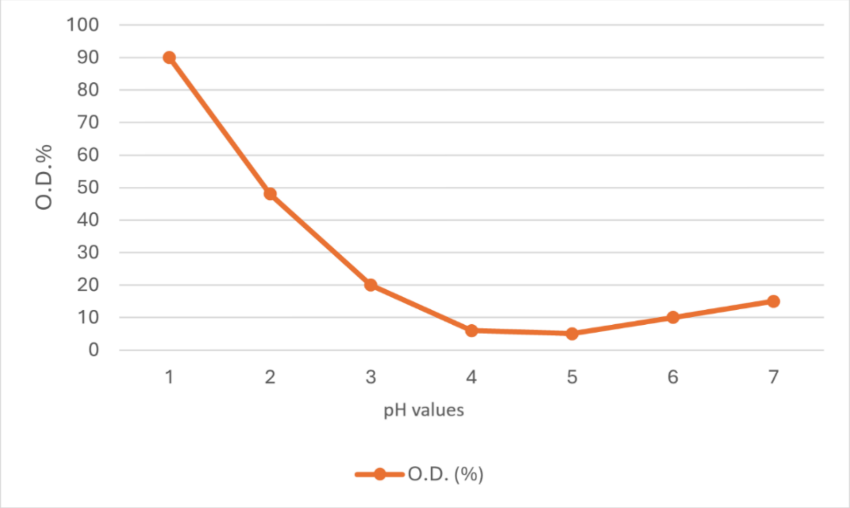


Fig. 2. Change in optical density O.D. λ_{max} 290 nm of D-glucose-glycine model solutions as function of pH (0.5 mol/l of each compound, H_2O, 4 hours)

To evaluate the influence of pH (from 7.0 to 9.0) and reheating (heating: 95 °C; reheating 6 hours) on the α-DCs formation, D-glucose-glycine model solutions (sample 2) were studied. α-DCs formed at both each pH and reheating time were determined based on the changes in the absorption of UV (λ_{max} 270–285 nm).

Based on the analysis of the intensities of carbonyl absorption, it can be seen that heating time accelerates the α-DCs formation to a greater extent in the pH range between 8.0 and 9.0 (Fig. 3).

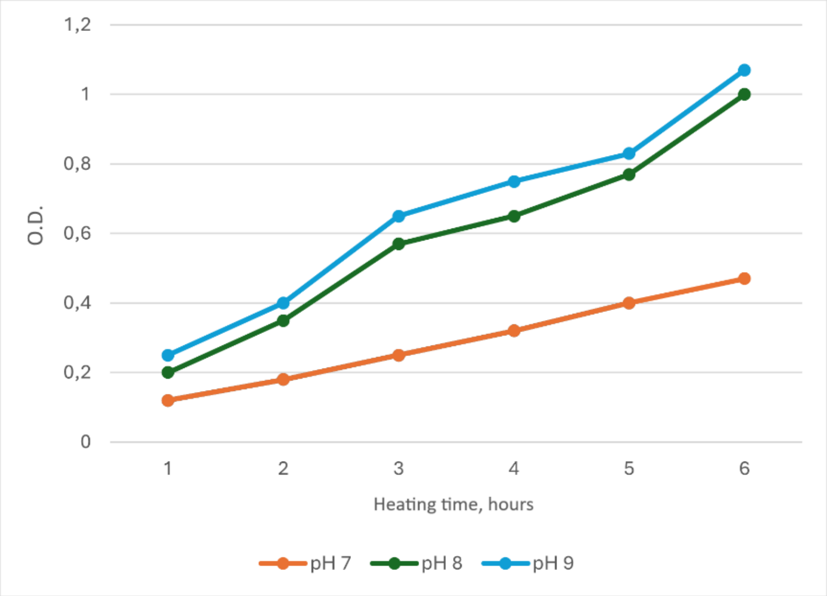


Fig. 3. **Change in optical density O.D. λ_{max} 270–285 nm of D-glucose-glycine model solutions as function of extended heating (0.5 mol/l of each compound, H_2O, 6 hours)**

To compare the effect of pH values (from 7.0 to 13.0) on the α-DCs formation in the D-glucose-glycine model system, the changes in the optical density (O.D.) of DCs were analyzed.

After reheating (heating: 95 °C; reheating 4 hours) of D-glucose-glycine model solutions (sample 2), the α-DCs formation at each pH was determined by UV absorption (λ_{max} 270–285 nm).

The difference in the effect of pH on the α-DCs formation made possible to identify different zones on the pH scale (Fig 4):

- in the pH range between 7.0 and 10.0, the carbonyl absorption intensity increased as the pH increased;
- in the pH range between 10.0 and 12.0, the carbonyl absorption intensity rapid increased as the pH increased
- in the pH range between 12.0 and 13.0, the carbonyl absorption intensity rapid decreased as the pH increased

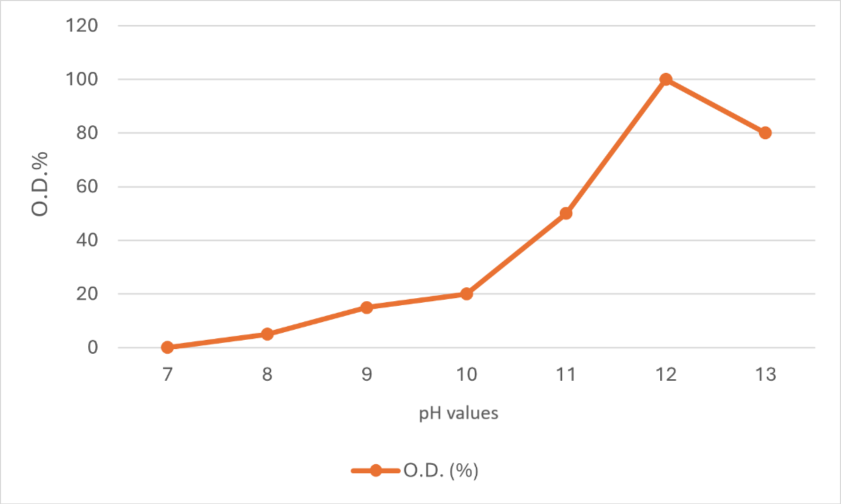


Fig 4. **Change in optical density O.D. λ_{max} 270–285 nm of D-glucose-glycine model solutions as function of pH (0.5 mol/l of each compound, H_2O, 4 hours)**

The obtained results are in agreement with calculations of the relative rates ($\upsilon = \frac{O.D.}{min.}$) of α-DCs formation in the D-glucose-glycine model system.

Table

Relative rates of α-DCs formation in the D-glucose-glycine model system
(0.05 mol/l D-glucose and glycine, H2O, 95°C, 4 h)

pH (-7.0)	1.0	2.0	3.0	4.0	5.0	6.0	7.0
$\upsilon = \frac{\text{O.D.(290 nm)}}{\text{min.}}$	0.0037	0.0008	0.0005	0.0003	0.0004	0.0007	0.0012
pH (7.0-13.0)	7.0	8.0	9.0	10.0	11.0	12.0	13.0
$\upsilon = \frac{\text{O.D.(275 nm)}}{\text{min.}}$	0.0012	0.0040	0.0233	0.1638	0.3250	0.3555	0.2833

The calculated data in quantitative terms allow us to compare the rates of α-DCs formation in the D-glucose-glycine model system (Table). It is quite obvious that in the pH range between 4.0 and 5.0 the formation of α-DCs occurs at the lowest rate.

The rates of α-DCs formation rapid increased as the pH increased from 10.0 to 12.0 and then decreased with a further increase of pH to 13.0.

In order to record the effect of reheating time (heating: 95 °C; reheating: 95 °C, 15 min, 3 h, 6 h) on the α-DCs formation, the D-glucose-glycine model solutions (sample 2. pH 9.0) were studied using Raman spectroscopy.

In the Raman spectra of the D-glucose-glycine solution (sample 2) at 20 °C the characteristic absorption peaks of the carbonyl group (1730, 1715 cm^{-1}), enol (1695 cm^{-1}) and enediol (1655 cm^{-1}) structures and peak of deformation vibrations of the N-H bond near 1645 cm^{-1} were observed.

After 15 min of heating the reaction mixture the stretching vibration band of the carbonyl group C=O occupies its usual position (1710 cm^{-1}) with low intensity, but is slightly shifted, the intensity of the enediol absorption (1655 cm^{-1}) has greatly increased, the band of deformation vibrations of the N-H bond (1645 cm^{-1}) has remained in its place, and the enol absorption band has disappeared.

After 3 hours of heating the reaction system, the frequency positions of characteristic bands changed and new absorption bands appeared:

- the band at 1730 cm^{-1} corresponds to the stretching vibration band of the carbonyl group C=O in α-DCs with medium intensity;
- bands in the region (1680–1620 cm^{-1}) are due to the stretching vibration band o of isolated C=C bonds
- the band of 1630 cm^{-1} can be attributed to t the stretching vibration band of the carbonyl group C=O in tert-amide-type structures.

After 6 heating hours of the D-glucose-glycine reaction system, only three absorption bands remain in the region of 1750–1600 cm^{-1}:

- the intensity of carbonyl absorption (1740 cm^{-1}), which corresponds to the stretching vibration band of the carbonyl group C=O in α-DCs has greatly increased;
- 1662 cm^{-1}, which can be attributed to the stretching vibration band of C=C bonds;
- in the amide region (about 1635 cm^{-1}); this could be related to the generation of typical Maillard reaction products (i.e., a Schiff base imine group and enaminol group) and the formation of covalent bonds between carbonyl group and amino group

Changes in the characteristic absorption bands of carbonyl (ν 1740–1710 cm^{-1}) in the D-glucose-glycine model solution depending on the temperature and reheating time at pH 9.0 are correlated with UV spectral studies.

Conclusion

The purpose of this study was to investigate the dynamics of the α-DCs formation in the D-glucose-glycine model system at both different pH values and extended heating based on the changes in UV absorbance at λ_{max} 270–290 nm. The obtained results supported those of previous studies that revealed the great effect of pH value on the intermediate products formation in the Maillard reaction. It was shown that during the heating time in the pH range between 4.0 and 5.0, the slowest α-DCs formation was observed. A comparative assessment of the effect of pH (from 1.0 to 7.0) on the α-DCs formation confirmed that in the pH range from 4.0 to 5.0, the α-DCs formation slows down to a greater extent. The α-DCs formation was accelerated in the pH range from 7.0 to 12.0, but nevertheless, in the pH range between7.0 and 10.0 less intense α-DCs formation was observed. The obtained results are in agreement with calculations of the relative rates ($\upsilon = \frac{O.D.}{min.}$) of α-DCs formation in the D-glucose-glycine model system.

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