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The Stability Aspects of Prepared cis isomers of lutein

Barbora Hrvolová[1], Jiří Kalina[1,2]
[1]*Department of Chemistry, Faculty of Science, University of Ostrava, Ostrava*
[2]*Environmental Center, Faculty of Science, University of Ostrava*

Abstract: The carotenoids, which also include lutein, are simple prenyl lipids. Their carbon skeleton consists of isoprenoid units that form long aliphatic conjugated systems of double bonds. Lutein can create geometrical isomers, all-trans and cis forms, due to the presence of delocated double bonds. Their different structures result in different physical and chemical properties. Due to their properties the cis isomers potentially have a higher antioxidant effect. All-trans forms are generally considered to be more stable than cis forms.

The main aim of this work was to present a possible use of isomers as a potentially improved antioxidant, and therefore we investigated their thermal stability. During exposure to 36°C and 40°C we observed a decrease in the all-trans form and an increase in both lutein cis forms. The first cis isomer was created with a higher probability than the second cis isomer. We observed the almost the same behaviour during the exposure to 6°C. The cis forms remained stable at 36°C and 40°C for a period of one hour and at 6°C for a period of 5 days. The quantity of cis forms rose at the expense of the all-trans form.

Keywords: *Thermal stability; Lutein; Isomers; High performance liquid chromatography*

I. INTRODUCTION

The carotenoids, which also include lutein, are simple prenyl lipids. Their carbon skeleton consists of isoprenoid units that form long aliphatic conjugated systems of double bonds. The length of a conjugated system is one of the most important properties of carotenoids. This property predetermines the spectral properties of a molecule and thus its biological function. The ends of polyene chains are usually twisted to form closed circles. Carotenoids can be divided into carbon derivates, carotenes, and oxygen derivates called xanthophylls. [1]

Lutein with its hydroxyl groups belongs among the xanthophylls. Lutein is hydrophobic and soluble in fats and lacks provitamin activity, like all xanthophylls. Lutein can be found in both animal and plant tissues. It has several important functions in organisms, such as light harvesting, pigmentation, and also photoprotective, antioxidant, and chemoprotective functions [2, 3]. It also participates in intracellular communication and immune response. At present, the most discussed property of lutein is its preventive effect against age-related macular degeneration (AMD) [4]. Since the preventive effect of lutein was first mentioned, significant progress has been recorded in medical research. At present, there is strong evidence of lutein's effect on protecting the eye against age-related macular degeneration [5-7]. Due to the presence of delocated systems of double bonds, carotenoids can create geometrical isomers, called stereoisomers, both all-trans and cis. Their different structures result in different physical chemical properties [8]. We observed higher absorption in the absorption spectrum of cis isomers around 340 nm (the cis peak), together with a shift towards shorter wavelengths [9].

Lutein is usually used in food supplements. In these supplements lutein is represented in its all-trans form, which has some antioxidant properties [10, 11]. Due to their physical chemical properties, cis isomers potentially have a higher antioxidant effect [11]. The availability and bioactivity of cis isomers in food is higher than that of the all-trans form [11]. Due to the high reactivity of carotenoids, their stability has been the subject of many studies [12-17]. Light [12], heat [18] and interactions with proteins or lipids [19] can affect the stability of carotenoids. Recent studies focusing on the stability of carotenoids include [11, 15]. These studies describe the stability of lutein in its all-trans form or in general. The aim of this work is to describe the behaviour of prepared cis isomers under certain thermal conditions.

II. MATERIALS AND METHODS

2.1 Preparation of lutein cis isomers

The basic solution of lutein was prepared by dissolving 1.2 mg of standard lutein (Mr = 568.87, Sigma-Aldrich, Germany) in 50 ml of 100% ethanol. The basic solution of iodine was prepared by dissolving 6.6 mg of iodine in 25 ml of 100% ethanol. 700 µl of the basic solution of lutein, 300 µl of n-hexane and 10 µl of the basic solution of iodine were mixed in a vial. The samples were irradiated by a special illuminating device [20] for one minute to create cis isomers that were detected by the HPLC system (HPST Agilent 1200, USA).

2.2 Thermal conditions and time exposure

The stability of cis isomers was tested at different temperatures, i.e. 6°C, 36°C and 40°C. The period of exposure was between 15 and 60 minutes at 36°C and 40°C. At 6°C the period of exposure was between 24 and 240 hours. The samples for stability verification at 36°C and 40°C were tempered in a dark place (an HPLC system autosampler). The samples for stability verification at 6°C were tempered in a dark place (a refrigerator).

2.3 Identification of lutein cis isomers

The HPLC system (HPST Agilent 1200, USA) with a Zorbax SB-C18 column (4.6 x 150 mm, 5 µm, Agilent, USA) and with a DAD detector was used for identification and quantification of lutein isomers. The combination of two mobile phases in the gradient configuration was used to analyze the lutein isomers. The first mobile phase consisted of methanol and water in the ratio of 85:15, v/v. The second phase contained methanol and n-hexane in the ratio of 4:1, v/v. A flow gradient in the mobile phase was used during the analysis. The flow ranged from 2ml/min to 0.5 ml/min. The column was tempered to 20°C. The detection occurred at wavelengths 270, 340 and 440 nm. The all-trans and cis lutein forms were identified on the basis of different spectral properties. An increased absorption at around 340 nm (cis peak) and a shift of main maximums towards shorter wavelengths were detected in the cis forms.

III. RESULTS AND DISCUSION

3.1 Evaluation of stability of lutein at 36 °C, at 40 °C and at 6 °C

Previous studies [11, 15] focusing on the thermal stability of lutein generally claim that with increasing duration of exposure and with increasing temperature the quantity of lutein decreases, especially the all-trans form of lutein, and the quantity of products of degradation increases. Lutein cis forms are usually considered as products of the degradation process. Our results are indirectly confirmed the findings of these studies.

The decrease in the quantity of the all-trans form and the increase in the quantities of both cis lutein forms (cis1, cis2) were observed during the exposure to heat at 36°C (Fig.1). The quantity of the all-trans form decreased during the whole time of exposure. The quantity of the all-trans form decreased by 30%. The quantity of the first cis form (cis1) rose during the first 45 minutes of exposure to 36°C. During the last 15 minutes of exposure the quantity was steady. The quantity of the cis1 isomer grew by 50% after 60 minutes' exposure. The quantity of the second cis isomer (cis2) rose during the first 15 minutes of the exposure to 36°C and remained steady until the end of exposure. The quantity of the cis2 isomer grew by 30% during the exposure.

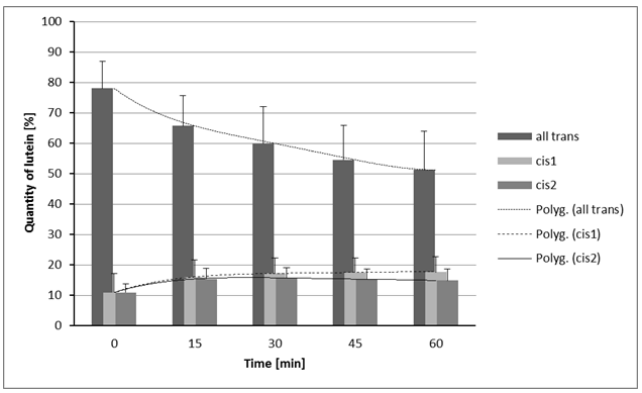

Fig. 1. Percentage representation of the quantity of the all-trans form and cis forms of lutein at 0, 15, 30, 45 and 60 minutes at 36°C. The columns represent the mean values + SD (n=20).

The decrease in the quantity of the all-trans form and the increase in the quantities of both cis lutein forms (cis1, cis2) were observed during exposure to heat at 40°C (Fig.2). The quantity of the all-trans form decreased during the whole time of exposure. The quantity of the all-trans form decreased by 30%. The quantity of the cis1 isomer increased during the whole time of exposure. The quantity of the cis1 isomer increased by 50%. The quantity of the second cis form (cis2) rose during the first 45 minutes of exposure to 40°C. During the last 15 minutes of exposure a small decrease in the quantity of the cis2 isomer was observed. The quantity of the cis2 isomer grew by 100%. The first cis isomer (cis1) originates with a higher probability than the second cis isomer (cis2) (Fig.1., 2.). The data were analyzed using one-way ANOVA and Tukey's comparison test. Significant differences between quantities of all-trans and cis forms at individual times on significance level $P < 0.05$ were shown by means of one-way ANOVA. Tukey's comparison test confirmed a significant decrease in the quantity of the all-trans form and a significant increase in the quantities of both cis forms on significance level $P < 0.05$ in comparison with the quantities of individual forms at zero time. Significant differences

between the results of 30, 45 and 60 minute exposures were not observed in all cases, which could indicate saturation behavior.

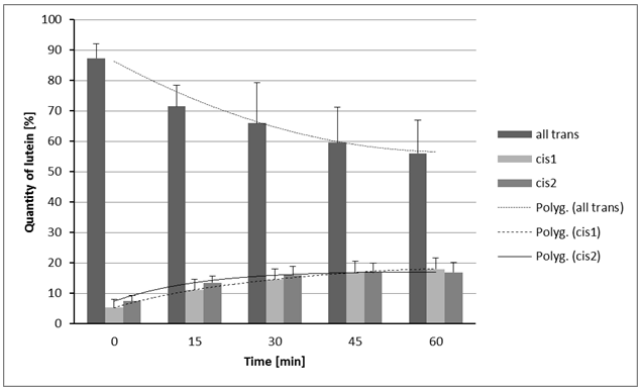

Fig. 2. Percentage representation of the quantity of the all-trans form and cis forms of lutein at 0, 15, 30, 45 and 60 minutes at 40°C. The columns represent the mean values + SD (n=10).

The thermal stability of all-trans and cis lutein forms at 6°C was measured in order to determine storage conditions. In this case, a significant increase in the quantity of the all-trans form was observed following 24 hours of exposure after the preparation of the mixture (Fig. 3). This phenomenon can be explained by the reconstruction of the broken fragments of lutein, which remained in the sample after the preparation of the mixture of cis isomers. For other lengths of exposure (48, 120 and 240 hours) a decrease in the quantity of the all-trans form was observed; the quantity of the all-trans form decreased by 10%. The quantity of the first cis form (cis1) rose during the first 120 hours of exposure to 6°C. A small decrease in the quantity of the cis1 isomer was observed at the end of exposure. The quantity of cis1 grew by 500 % during the exposure. The quantity of the second cis form (cis2) rose during the whole time of exposure to 6°C. The quantity of cis2 grew by 300 % during the exposure. These data were also evaluated by means of one-way ANOVA and Tukey's comparison test. On significance level $P < 0.05$ one-way ANOVA showed significant differences between the quantities of all-trans and cis forms at individual times. Tukey's comparison test confirmed a significant decrease in the quantity of the all-trans form, with the exception of an increase between time 0 and 24 hours, and a significant increase in the quantity of both cis forms compared with the quantity at time 0 on significance level $P < 0.05$. Significant differences between the results of 48, 120 and 240 hour exposures were not observed in all cases, which could indicate saturation behavior.

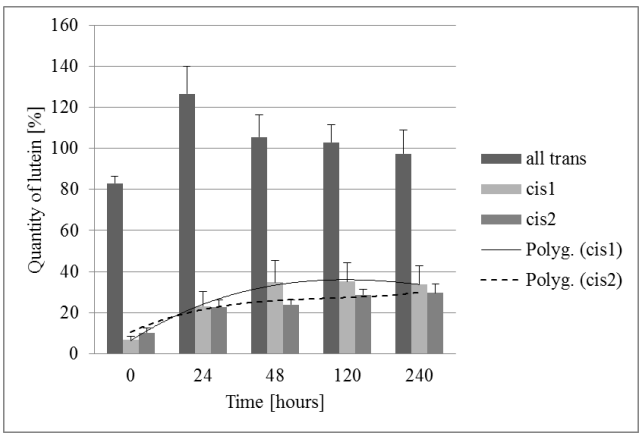

Fig. 3. Percentage representation of the quantity of the all-trans form and cis forms of lutein at 0, 24, 48, 120 and 240 hours at 6°C. The columns represent the mean values + SD (n=5).

IV. CONCLUSION

The results of this study indicate that the quantity of the all-trans form decreases, with the exception of exposure to 6°C for 24 hours, and the quantity of cis forms increases as the time of exposure increases at different temperatures. The quantity of the all-trans form decreases by 30% after 60 minutes' exposure to 36°C. The quantity of the cis isomer (cis1) increases by 50% and the quantity of the cis isomer (cis2) increases by 30% after 60 minutes' exposure to 36°C. The quantity of the all-trans form decreases by 30% after 60 minutes' exposure to 40°C. The quantity of the cis isomer (cis1) increases by 150% and the quantity of the cis isomer

(cis2) increases by 100% after 60 minutes' exposure to 40°C. The quantity of the all-trans form decreases by 10% after 240 hours' exposure to 6°C. The quantity of the cis isomer (cis1) increases by 500% and the quantity of the cis isomer (cis2) increases by 300% after 240 hours' exposure to 6°C. In conclusion, our results show that more extreme conditions lead to a decrease in the quantity of the all-trans form and an increase in the quantity of cis forms.

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