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# THE ROLE OF LIGHTENING, THERMAL ENERGY, AND NATURE OF THE SOLVENT ON TORULARHODIN DEGRADATION

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## ABSTRACT

Investigation into the potential health benefits of the carotenoid torularhodin has increased in recent years, mainly due to its strong antioxidant activity, which is higher than other counterparts such as β-carotene and lycopene. However, torularhodin has not yet been commercially produced, and research on this topic lacks crucial information on its stability under different processing conditions. These data are essential for accelerating the entry of this compound into the market. In this study, the effect of light exposure, thermal energy, and solvent type on the degradation of torularhodin was investigated. A torularhodin-rich extract produced by the yeast *Rhodotorula glutinis* was used. The photodegradation and thermodegradation (at 4, 25, and 45 ºC) were analyzed in two different bio-based solvents, namely ethanol and ethyl acetate. The stability of torularhodin in both solvents was negatively affected by light exposure. When torularhodin is exposed to light for a short period of time (less than 24 h), it degrades in ethyl acetate but does not degrade in ethanol. There was no significant difference in the preservation of stability at temperatures of 4 and 25 ºC for both solvents. This study demonstrates the importance of selecting an appropriate solvent and processing conditions for torularhodin stability.

**Keywords:** Carotenoid. Photodegradation. Stability. Bio-based Solvent.

## 1 INTRODUCTION

Torularhodin is a xanthophyll carotenoid pigment produced by microorganisms. Its structure is composed of a carbon-hydrogen chain with 13 conjugated doubles bonds, a non-cyclic β-ionone ring, and a terminal carboxylic group[1]. Recently, torularhodin has gained attention in academic research for pharmaceutical and nutraceutical applications due to its strong antioxidant activity, as well as anti-inflammatory, anti-microbial, and anti-cancer properties[2]. The health benefits of torularhodin demonstrated to date include reduction of chronic kidney disease caused by high-fat diets[3], reduction of oxidative stress, inflammatory reaction and apoptosis of hepatocytes in drug-induced liver injury[4], attenuation of cognitive impairment and neuroinflammation in neurodegenerative diseases [5], among others.

As with other natural pigments, a high-yield production and efficient application of torularhodin is highly dependent on its chemical and structural stability. The degradation or isomerization of carotenoids may result in a change or loss of their bioactivity properties. Carotenoids are generally unstable to reactive oxygen species, light, and heat [6]. From production to formulation, carotenoids are exposed to several damaging conditions. The intracellular character of carotenoids requires the rupture of the microorganism cell wall and laborious extraction procedures to release the molecule. Fossil-derived organic solvents are extensively used in this step and in the purification process due to the lipid-soluble nature of carotenoids. However, safer and more sustainable solvents derived from renewable sources (bio-based solvents) have been used as greener substitutes due to the well-known risks of traditional solvents to human health and the environment [7]. Ethanol and ethyl acetate (EtAc), among other bio-based solvents, exhibit high solubility and are effective in extracting carotenoids and other natural compounds.

In this sense, understanding the appropriate conditions for working with torularhodin is necessary to contribute to its successful production, application, and future entrance in the market. Therefore, this study investigated how the stability of microbial torularhodin, produced by the red yeast *R. glutinis*, is affected by the thermal energy, light exposure, and interaction with two of the most bio-based solvents used in the carotenoids processing.

## 2 MATERIAL & METHODS

Thermal and photodegradation studies of torularhodin in bio-based solvents were performed with a torularhodin-rich extract obtained from the red yeast *R. glutinis*. The extract was obtained using a modified method based on MUSSAGY et al. (2021) [8]. The torularhodin-rich extract was separately solubilized in ethanol and EtAc (2 mg/mL) and incubated under different temperature and light exposure conditions. The carotenoid thermal decomposition was investigated at 4, 25, and 45 ºC for 312 h in complete absence of light. To evaluate photodegradation, the samples were exposed to artificial light (1220 lx) at room temperature (25 ºC) for 456 hours. Since the EtAc samples showed rapid decomposition, the extract solubilized in this solvent was exposed to more intense light (3420 lx) for 24 h to be analyzed over shorter periods of time. Torularhodin degradation was determined by measuring the absorbance of the sample at the wavelength of maximum absorption ($\lambda_{max}$ = 485 nm), as exhibited by the torularhodin extract scan analysis at the beginning of the experiment. The results were presented in terms of Stability (%), determined by Eq. ($A/A_0$) *100, where $A$ is the sample absorbance and $A_0$ is the sample absorbance at time 0, i.e., at the outset of the assay. One-way analysis of variance (ANOVA) was performed using Origin software to test for significant difference between means using Tukey's multiple comparison test. Data were statistically significant when $p < 0.05$.

## 3 RESULTS & DISCUSSION

Preserving the chemical structure and bioactive properties under external conditions is fundamental for the successful application of natural products. Some of the most well-known carotenoids, such as beta-carotene and astaxanthin, have already been the subject of numerous studies investigating their stability. To gain further insight into the properties of torularhodin, its degradation when solubilized in ethanol and EtAc was studied under light (Figure 1) and heat exposure (Figure 2).

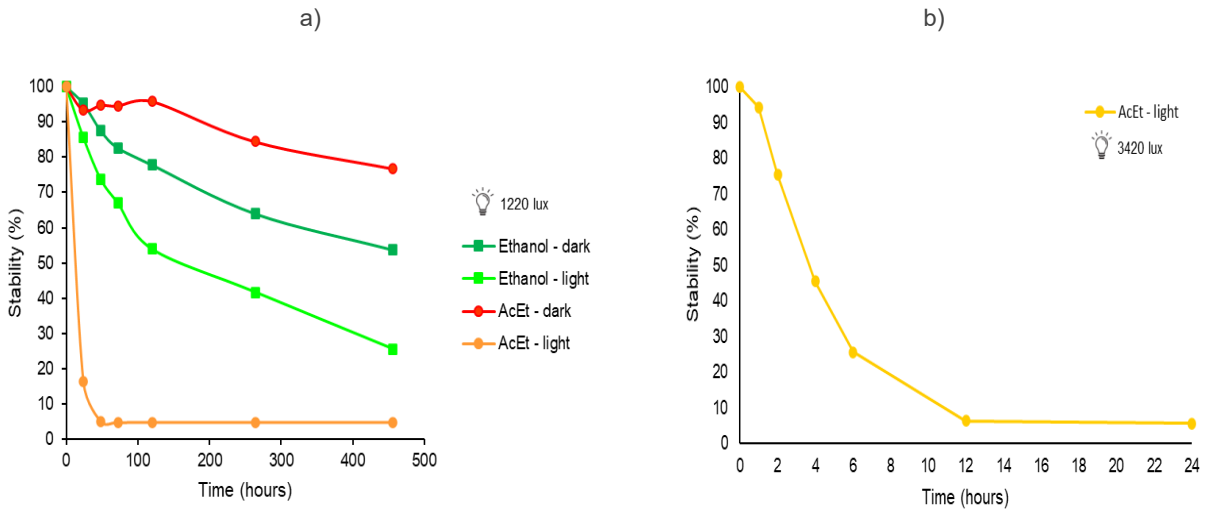


**Figure 1**. Stability of the torularhodin-rich extract in a) ethanol and ethyl acetate (AcEt) under exposure to 1220 lux, and b) in AcEt under exposure to 3420 lux.

The effect of lightening in the stability of the torularhodin-rich extract at 25 ºC differed between the two bio-based solvents, ethanol and EtAc, as shown in Figure 1-a). In the absence of light incidence, the carotenoid exhibited higher stability in both solvents than in the light condition. However, after 24 hours, the stability of torularhodin diluted in ethanol in dark condition (dark green) starts to decrease faster than in AcEt (dark red) in the same condition. After 456 hours, the stability of ethanol-sample decreased by approximately 50%, while the stability of AcEt-sample remained around 76%. The degradation pattern changed when the samples were exposed to light incidence. The light-induced degradation in ethanol-sample (pale green) was more pronounced during the first 120 hours, when the carotenoid had reached about half of its stability (53%). The degradation rate then decreased, but the carotenoid continued to lose stability, reaching 25% after the next 336 hours. In contrast, AcEt-sample exhibited a stronger negative effect under light exposure (pale red). The incidence of 1220 lux resulted in almost completely degradation of the carotenoid, which reached a stability of 16% in 24 hours and 5% in 48 hours.

The degradation pattern of the AcEt-sample within 24 hours was then evaluated for a light incidence of 3420 lux (Figure 1-b). As shown, torularhodin-rich extracts solubilized in AcEt suffered a rapid photodegradation. In the first 4 hours, carotenoid stability decreased by more than 50%, and up to 12 hours, approximately 95% of the carotenoid stability was lost. A similar rapid degradation was observed for astaxanthin, another xanthophyll carotenoid, upon dilution in AcEt and exposure to light-emitting diode (LED) irradiation [10].

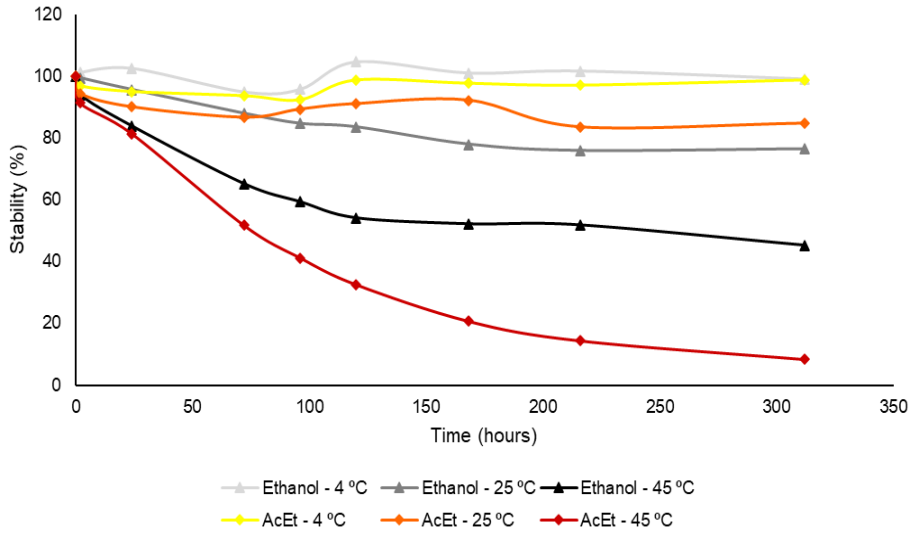


**Figure 2** Torularhodin-rich extract under temperatures of 4, 25, and 45 ºC in ethanol and ethyl acetate.

The degradation of torularhodin-rich extract induced by thermal energy at 4, 25, and 45 ºC under dark conditions is shown in Figure 2. It can be observed that torularhodin, when solubilized in ethanol and AcEt, maintains its complete stability for a period of 312 hours when stored at 4 ºC. A slight degradation of torularhodin was observed when it was stored at 25 ºC. Nevertheless, no significant difference was observed in the extent of degradation between 4 and 25 ºC. However, when the temperature was increased to 45 ºC, there was a decline in torularodine stability within the initial exposure period. As it can be noted, the torularhodin degradation at 45 ºC ocurred for both Ethanol and EtAc extracts. High temperatures have already been shown to be an important factor influencing the decomposition of the chemical structure of carotenoids. The presence of conjugated double bonds renders carotenoids susceptible to oxidation and isomerization in the presence of elevated temperatures and light exposure, as well as other environmental factors [4]. In addition, it is already known that the isomers formed in thermal isomerization and photoisomerization process have different structures and properties and, consequently, different stabilities [10]. As a result, maintaining temperatures between 4 and 25°C was found to be more beneficial in limiting the isomerization reaction, thus preserving the structural and physical properties of torularhodin.

## 4 CONCLUSION

The incidence of light and exposure to 45°C have a negative effect on the stability of the torularhodin-rich extract. A thermo-decomposition of the extract was significant at 45 ºC in ethanol and EtAc. Incubation under light resulted in faster and stronger degradation of the extracted solubilized in EtAc than in ethanol. Commonly, solvents are selected based on their ability to solubilize and extract the target molecule. However, these findings highlight the importance of understanding how a specific solvent can interact with a specific molecule. Further studies to understand the mechanisms involved in the interaction of torularhodin with other bio-based solvents are needed to support the implementation of these solvents in this carotenoid recovery.

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