Raman Spectroscopy of Carotenoids in *Sarcoscypha coccinea* (Fungi)

Volodymyr Dzhagan[1,2], Yuriy Azhniuk[3,4], Veronika Dzhagan[2], Oleksandr Hreshchuk[1], Halyna Azhniuk[5], Vasyl Lopushansky[4]

[1] *V. Lashkaryov Institute of Semiconductor Physics, Nat. Acad. Sci. Ukr., Kyiv, Ukraine*
[2] *Taras Shevchenko National University of Kyiv, Kyiv, Ukraine*
[3] *Institute of Electron Physics. Nat. Acad. Sci. Ukr., Uzhhorod, Ukraine*
[4] *Uzhhorod National University, Uzhhorod, Ukraine*
[5] *Z.O. School 7, Uzhhorod, Ukraine*

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INTRODUCTION

Many species of fungi are known to produce carotenoids whose protective role against oxidative stress and exposure to visible UV light has been shown.[1]. The most abundant carotenoid in nature, β-carotene, was reported in many ascomycete fungi, including species from the Pezizales order [2].
Sarcoscypha coccinea (Jacq.) Sacc. (Scarlet elf cup) is an operculate cup fungus from the order Pezizales (Ascomycota) which forms large, bright scarlet fruitbodies with large, smooth, almost cylingrical spores in 8-spored, inamyloid asci. The bright colored hymenium of S. coccinea, as well as of the related ascomycete fungi, are known to be due to high content of carotenoids [3]. Carotenoids reported so far to be contained in S. coccinea are β-carotene, plectaniaxanthin and its derivatives, such as dehydroplectaniaxanthin, γ-carotene, and torulene [3,4]. However, the detailed carotenoid composition of the ascomycetes fungi in general and *Sarcoscypha coccinea* in particular has been rarely investigated so far.
Here we present a study of carotenoids in *Sarcoscypha coccinea* by Raman spectroscopy trying to show the advantages of this fast and non-destructive technique over high-performance liquid chromatography, thin layer chromatography, and column chromatography which are the most popular characterization techniques used for establishing the carotenoid composition [5].

MATERIALS AND METHODS

Samples of scarlet elf cup, a fungus belonging to the Sarcoscyphaceae family of the order Pezizales, were collected in two areas of Ukraine (a mixed forest in the city of Kyiv and in a mixed submountain forest in Carpathians near Uzhhorod).
Raman spectra were measured from as-collected specimen at room temperature using a XPloRa Plus spectrometer (Horiba) and a modified MDR-23 spectrometer (LOMO) with the excitation by a solid-state laser (532 nm). The iinstrumental resolution was better than 5 cm^{-1}.

Fig. 1. Fruitbodies of *Sarcoscypha coccinea* collected in Kyiv area (a) and Uzhhorod area (b)

RESULTS AND DISCUSSION

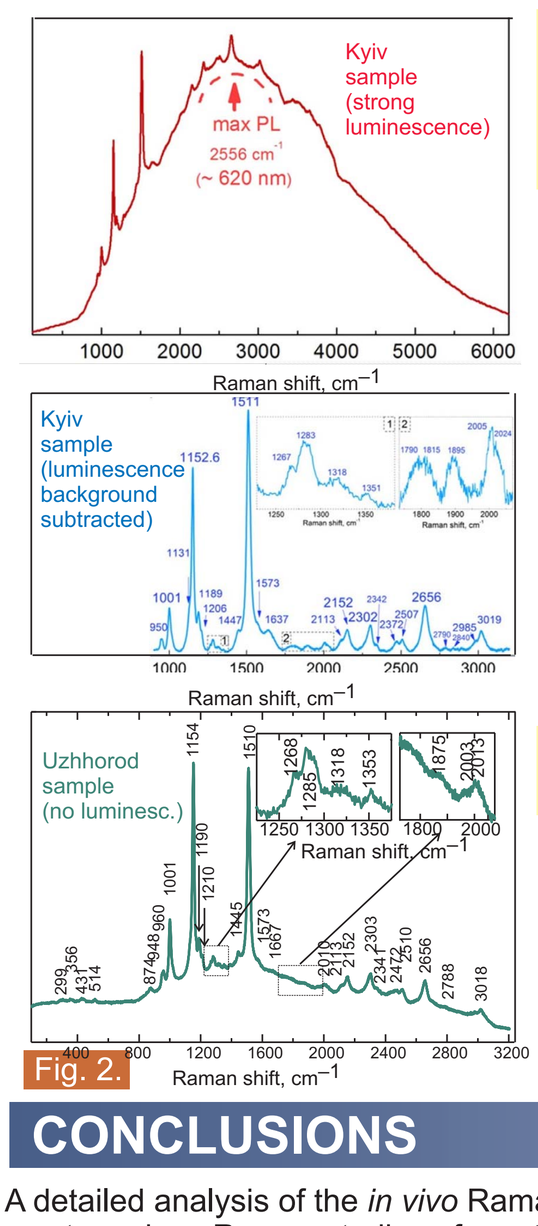

Fig. 2.

Raman spectra of the Kyiv sample are observed at a background of an intense broad photoluminescence (PL) band centered at 620 nm. The Uzhhorod sample exhibits practically no PL signal. However, the spectrum of the Kyiv sample with subtracted PL background is practically identical to that of the Uzhhorod sample.

The peaks at 1001, 1152, and 1511 cm^{-1} match well the characteristic set of peaks commonly reported for carotenoids: 1000–1020 cm^{-1} (ν_3), 1150–1170 cm^{-1} (ν_2), and 1500–1550 cm^{-1} (ν_1). These modes are often used for identification of the types of carotenoids. The (ν_1) mode is most sensitive to the length of conjugated (polyene) chain; the (ν_2) band is actually constituted of contributions from stretching vibrations of C–C single bonds coupled with C–H in-plane bending modes, and this region is a fingerprint for the assignment of cis-isomers; (ν_3) arises from in-plane rocking vibrations of the methyl groups attached to the conjugated chain, which are coupled with in-plane bending modes of the adjacent C–H's and can be used as a fingerprint for the configuration of conjugated end-cycles [6].

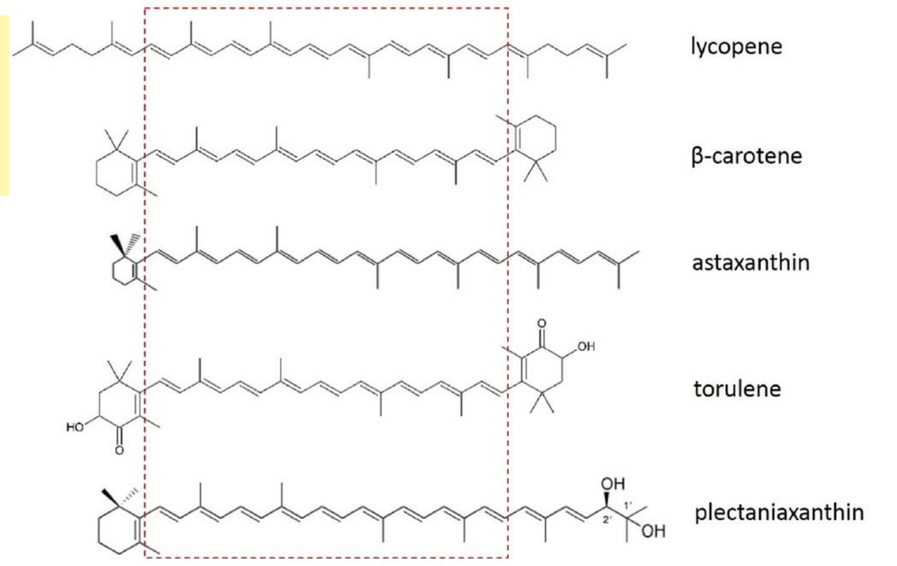

Fig. 3. Schematic structure of the carotenoid molecules potentially present in *Sarcoscypha coccinea*. The red short-dashed line marks the common part of the molecule.

Possible external factors that can affect the Raman peaks and therefore their interpretation:
❶ environment, ❷ bonding, ❸ excitation wavelength

Our approach to the analysis of the obtained Raman spectrum of *Sarcoscypha coccinea* (Fig. 2) and its attribution to certain type of carotenoids:
❶ as most probable candidates, we considered those carotenoids that were suggested from earlier investigations by other methods: β-carotene, plectaniaxanthin, and its derivatives, and γ-carotene;
❷ we considered matching of as big as possible number of vibrational modes in our spectra of *Sarcoscypha coccinea* to those in the literature spectra of carotenoids, both in pure (molecular) state and at different *in vivo* conditions;
❸ we analyzed all the available Raman studies of carotenoids in other fungi, to take into account possible common features

CONCLUSIONS

A detailed analysis of the *in vivo* Raman spectrum of *Sarcoscypha coccinea* in this work allowed all the vibrational bands to be attributed to carotenoids. Contrary to most previous Raman studies of carotenoids, focused on three spectral features near 1000, 1150, and 1500 cm^{-1} , we resolved and analyzed 30 vibrational bands, including rarely studied range above 2000 cm^{-1} . Carotenoid composition suggested from our spectroscopic analysis generally supports the conclusions of few earlier studies of *Sarcoscypha coccinea* by other methods. In particular, we suggest β-carotene, plectaniaxanthin (and its derivatives, such as dehydroplectaniaxanthin), γ-carotene, astaxanthin, and torulene as possible carotenoid species in this fungi. Importantly, we suggest two characteristic ring vibrations, at 1131 and 1283 cm^{-1} , to be used as a measure of the ratio of astaxanthin and plectaniaxanthin to β-carotene and lycopene in this and similar fungi.
The detailed Raman study of *Sarcoscypha coccinea* performed in this work is suggested to facilitate subsequent (more quantitative) studies of carotenoid composition in other cup fungi using Raman spectroscopy in combination with other techniques.

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