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Investigations on Carotenoids in Fungi
VI. Representatives of the *Helvellaceae* and *Morchellaceae*

By

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Zusammenfassung

Untersuchungen an Carotinoiden von Pilzen

VI. *Helvellaceae* und *Morchellaceae*

An den Fruchtkörpern von 14 Arten aus der Familie der Helvellaceen und von 3 Vertretern der Morchellaceen wurden säulen- und dünnschichtchromatographisch Vorkommen und Menge der Carotinoide bestimmt. Es wurden 28 Carotine gefunden. Weiters bestehen quantitative und qualitative Unterschiede im Carotinoidgehalt der Fruchtkörper von Helvellaceen und Morchellaceen. (Editor)

Summary

By means column and thin-layer chromatography the occurrence of carotenoids and their content was determined in fructifications of 14 species from the family *Helvellaceae* and 3 species from the family *Morchellaceae*. 28 carotenoids were found. Moreover quantitative and qualitative differences were found in the content of carotenoids in fructifications of *Helvellaceae* and *Morchellaceae* family.

Introduction

As I mentioned in my review of the literature on the occurrence of carotenoids in fungi (Czeczuga 1973), most carotenoids are either the provitamin of vitamin A or resemble that vitamin in their own biological activity (Bauernfeind 1972), hence the justifible interest in the carotenoid content in the fructifications of various fungi. Such studies carried out on different species of fungi can, as Valadon (1976) stated, be of value in

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taxonomic investigations. The fact that to date there is a lack of information on the carotenoid content of the species of fungi with which this paper deals (GOODWIN 1976, CZECZUGA 1973) has convinced us that the publication of the data we have obtained on the subject is warranted.

Material and Methods

The fructifications of *Helvella crispa* FR. ex SCOPOLI, *H. esculenta* FR. ex PERSOON, *H. gigas* (KROMBH.) COOKE, *H. infula* FR. ex SCHAEFFER, *H. lacunosa* AFZ. ex FR., *H. monachella* SCOP. ex FR., *H. pallescens* SCHAEFT., *H. pezizoides* AFZ., *Discina ancilis* (PERS.) SACC., *D. reticulata* (GREV.) SACC., *Disciotis venosa* (PERS. ex FR.) BOUDIER, *Leptopodia elastica* (BULL.) BOUD., *Paxina acetabulum* (L. ex FR.) KUN., *Rhizina inflata* (SCHAEFF.) QUEL., *Morchella conica* PERS. ex PERS., *M. esculenta* (L.) ex ST. AMANS and *Verpa digitaliformis* PERS. ex FR. were collected in 1973—1978 from the Knyszyn-Białystok Forest.

In the species investigated the carotenoids content was estimated in fructifications (pileus with stipe), while in *Verpa digitaliformis* they were measured in pileus and in the stipe, separately.

The fructifications were cleaned of all organic debris, macerated and placed into dark glass bottles and covered with acetone thus exchanging the air above the fluid in the bottle for nitrogen. The samples were kept in a refrigerator until removed for chromatographic analysis of the carotenoid content.

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at a temperature of about 20° C for 24 hours in the dark in a nitrogen atmosphere.

Columnar and thin-layer chromatography, described in detail in our previous papers (CZECZUGA 1978) were used for the separation of the various carotenoids. A glass column (Quickfit—England) approximately 1 cm ⌀. and 15—20 cm in lenght, filled with Al_2O_3, was used in column chromatography. The extract was passed through the column after which the different fractions were eluted with the solvent. Silica gel was used for the thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot. For identification of β-, γ-carotene, canthaxanthin, and astaxanthin co-chromatography was applied using identical carotenoids (Hoffmann — La Roche and Co. Ltd., Basle, Switzerland).

The pigments were identified by the following methods: a) behaviour on column chromatography, b) absorption spectra of the pigments in various solvents were recorded a Beckman spectrophotometer model 2400 Du, c) the partition characteristics of the carotenoid between hexane and 95% methanol, d) comparison of R_f on thin-layer chromatography, e) the presence of allylic hydroxyl groups was determined by the acid chloroform test, and f) the epoxide test.

Table 1

The carotenoid composition of the *Helvella* species: % of total carotenoids

Carotenoid	*H. crispa*	*H. esculenta*	*H. gigas*	*H. infula*	*H. lacunosa*	*H. monachella*	*H. pallescens*	*H. pezizoides*
neurosporene	10.1	11.6	18.8	9.5	52.9	11.8		
lycopene				3.8				
β-carotene	7.4	5.1	5.8	59.5	23.6	23.4		
γ-carotene						13.9		
ζ-carotene							16.5	
β-zeacarotene			12.1					
canthaxanthin		14.6		5.6				
β-cryptoxanthin			18.7	7.6				
aleuriaxanthin			3.5	1.0				
aleuriaxanthin ester			12.3					
astaxanthin	14.0	11.5						
flavoxanthin		4.3						36.8
lycoxanthin		3.0	1.1	2.7				
mutatochrome							28.3	29.5
neurosporaxanthin		14.4			11.9	16.6		
rubixanthin		12.1				34.3		
torularhodin			11.5					
3,4-dehydrolycopene	43.8	20.8	16.2				42.9	
hydroxy-ζ-carotene	8.2							
dihydroxy-ζ-carotene	11.2				11.6			33.7
1, 2, 1′, 2′-tetrahydro-1, 1′-dihydroxyly-copene		2.6		8.7				
unknown	5.3			1.6			12.3	

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations were based of the extinction coefficient E 1%/cm at the wavelengths of maximal absorbance in petroleum ether or hexane.

Results

The occurrence of the various carotenoids in the species of the *Helvella* genus is shown in Table 1 and that of the other species of *Helvellaceae* in Table 2. The chromatographic analysis revealed the presence of 6 carotenes and 18 xanthophylls in the *Helvellaceae* species under investigation. Neurosporene occurred in most of the species of *Helvellaceae*, β-carotene in only 7 species, whereas lycopene, γ-carotene, ζ-carotene and β-zeacarotene were

Table 2

The carotenoid composition of the species of the *Helvellaceae* family: % of total carotenoids

Carotenoids	*Discina ancilis*	*Discina reticulata*	*Discioths venosa*	*Leptopodia elastica*	*Paxina acetabulum*	*Rhizina inflata*
neurosporene	31.8	12.0		49.6	23.8	6.6
lycopene		9.5				
β-carotene					26.5	
astaxanthin				9.1		
capsanthin		7.4				
lycoxanthin		6.1			19.3	
mutatochrome	22.4	7.2		17.5		25.1
neurosporaxanthin			11.4		30.4	9.6
rhodopin		15.7				13.8
torularhodin			8.4			
hydroxy-ζ-carotene		7.3				
dihydroxy-ζ-carotene	19.1	3.6				
lycopene-5,6-epoxide	26.7	59.7				
1, 2, 1′, 2′-tetrahydro-1, 1′-dihydrocylycopene			20.5			33.0
unknown	31.2			23.8		11.9

noted only in single species. Most of the xanthophylls found in the *Helvellaceae* species investigated have previously been found in other fungi species. Six xanthophylls which were found in our material, however, rarely occur in fungi, that is canthaxanthin, aleuriaxanthin, astaxanthin, flavoxanthin, mutatochrome and plectaniaxanthin. The total carotenoid content of the *Helvellaceae* ranged from 0.025 μg/g (*Paxina acetabulum*) to 0.742 μg/g fresh mass (*Leptopodia elastica* Table 4).

The results of the analysis of the *Morchellaceae* species are presented in Table 3. As this table shows, in the fructification of the *Morchella conica*, 3 carotenes were found, including δ-carotene which was not noted in the *Helvellaceae* species. Five xanthophylls were also determined. In the fructification of the *Morchella esculenta*, 3 carotenes and 4 xanthophylls were found, including astaxanthin and zeaxanthin. Of the other species of the *Morchellaceae*, the *Verpa digitaliformis*, was found to contain 3 carotenes and 5 xanthophylls. The pileus of this fungus contained more carotenoids and their total content was greater. The stem of this species contained only 4 carotenoids and the total content was 0.065 μg/g whereas the pileus contained 6 carotenoids with a total of 2.132 μg/g fresh weight.

Table 3

The carotenoid composition of the some species of the *Morchellaceae* family: % of total carotenoids

Carotenoids	*Morchella conica*	*Morchella esculenta*	*Verpa digitaliformis* pileus	stipe
neurosporene	7.5	12.6	10.4	34.8
β-carotene		12.9	0.3	28.1
γ-carotene	5.3			
δ-carotene	1.7			
ζ-carotene		1.2		19.7
astaxanthin		22.2		
canthaxanthin			17.8	
lycoxanthin	6.1		36.8	
mutatochrome		12.1		17.4
mutatoxanthin	18.2			
plectaniaxanthin	10.1			
rubixanthin	15.7		16.3	
3,4-dehydrolycopene	15.7		11.1	
zeaxanthin		28.9		
unknown	19.7		7.3	

Table 4

Total carotenoid content in the some species of the *Helvellaceae* and *Morchellaceae* family ($\bar{x}$ = mean from 5 signatures, s = standard deviation)

Species	μg/g fresh weight ($\bar{x} \pm s$)
Helvellaceae	
Helvella crispa	0.668 ± 0.012
H. esculenta	0.091 ± 0.007
H. gigas	0.179 ± 0.011
H. infula	0.105 ± 0.005
H. lacunosa	0.190 ± 0.008
H. pallescens	0.229 ± 0.012
H. pezizoides	0.118 ± 0.005
Discina ancilis	0.180 ± 0.003
D. reticulata	0.187 ± 0.009
Leptopodia elastica	0.742 ± 0.019
Paxina acetabulum	0.025 ± 0.009
Rhizina inflata	0.216 ± 0.018
Morchellaceae	
Morchella esculenta	0.343 ± 0.012
Verpa digitaliformis	
pileus	2.132 ± 0.025
stipe	0.066 ± 0.008

Discussion

The commonest carotenoid in the material investigated was found to be neurosporene, which was present in most of the species of *Helvellaceae* and in both of the *Morchellaceae* species. As FIASSON & ARPIN (1967) and BONALY (1968) showed neurosporene is the precursor of lycopene, from which, after a series of conversions, γ-carotene and β-carotene are formed.

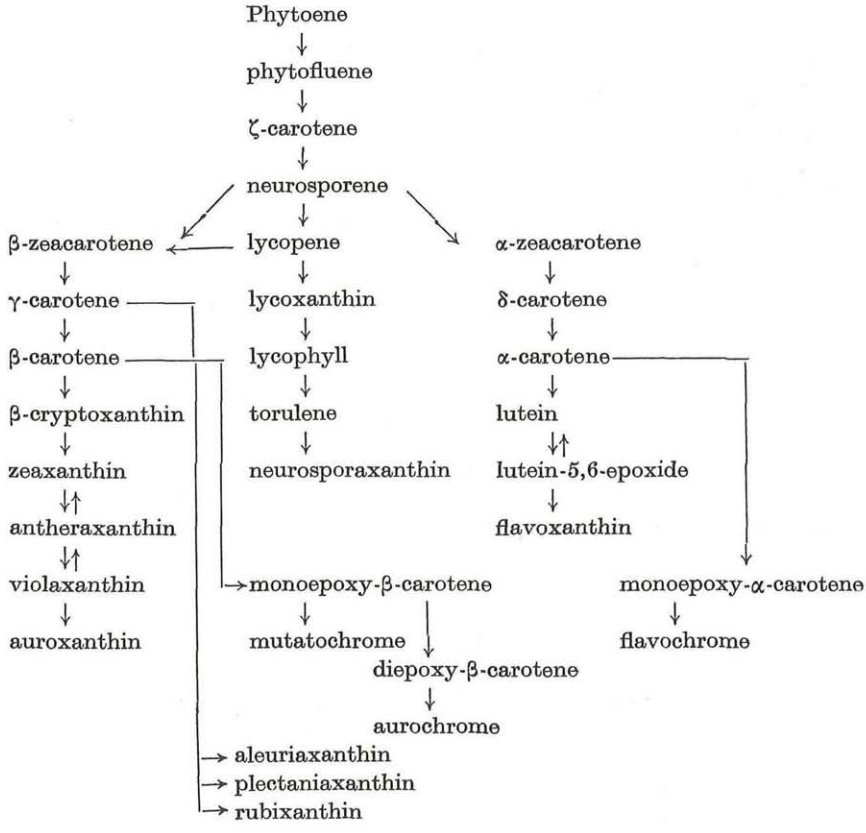

Fig. 1. Suggested pathaways of the synthesis of lycopene, α-, β-, and γ-carotene and of their derivatives (VALADON 1976)

Most of the carotenoids found in the species we investigated were lycopene derivatives γ- or β-carotene. The total carotenoid content of most of the *Helvellaceae* species was not particularly high. In the *Morchellaceae* specimens studied, considerable differences were found in the total carotenoid content of the different parts of the *Verpa digitaliformis*. While the stem contained only 0.066 μg/g fresh weight the pileus contained a comparatively large amount of carotenoid that is 2.132 μg/g fresh weight. A similar observation

was made in studies on the *Boletus luridus* (CZECZUGA 1978) and *Leccinum duriusculum* (CZECZUGA 1978), in which the pileus also contained more carotenoids than the stem. It is worth noting that several species were found to contain mutatochrome, its content ranging from 12.1% (*Morchella esculenta*) to 29.5% (*Helvella pezizoides*). In all probability, the presence of this carotenoid in our material was due to the ageing of the fructifications. As we know, mutatochrome is a derivative of β-carotene (5,8-dihydro—5.8-epoxy—β-carotene) and, as a number of experimental studies have shown (SIMPSON *et al.* 1976), it is formed in plant tissues as a result of the degradation of β-carotene. This may be the explanation for the presence of mutatochrome in the fructification of fungi in which β-carotene was not found to be present. The epoxide form of β-carotene (5,8-expoxide—β-carotene) was found in old cultures of the fungus *Phycomyces blakesleanus*, to be a degradation product (GOODWIN 1976). The content of these epoxide forms of β-carotene increases in ripening fruits of some plants (SIMPSON *et al.* 1976). The epoxide forms of β-carotene (mutatochrome, aurochrome and others) also occur in quite large amounts in lichens (CZECZUGA 1978).

The scheme of biosynthesis of carotenoids in plants (Fig. 1) shows its three pathways starting from neurosporene, (pathway β-zeacarotene, pathway lycopene and pathawy α-zeacarotene). The carotenoides found in the material studied are the result of metabolism of β-zeacarotene nad lycopene.

In conclusion it may be said that the differences found in the present investigations in the presence of the various carotenoids, particularly as concerns the dominant carotenoids in these species may be used in taxonomic studies of these fungi. It was also found that fungi with edible fructifications contained quite a fair amount of the biologically active compounds, carotenoids, which being the provitamins of vitamin A, enhance their nutritive value.

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