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Meeting on July 1, 1946

Title: Antioxidant properties of carotenoids and their derivatives.

Author: Armand HERISSET

Journal: Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, v.253, pp. 47-49 (July – December) 1946.

Plant Biology: Antioxidant properties of carotenoids and their derivatives. Note (1) by Mr. Armand Herisset, presented by Mr. Louis Blaringhem.

The plant cells of *Trentepohlia* Mart. and numerous chlorophyll cysts contain astaxanthin dissolved in lipid droplets. It is traditionally thought that the carotenoid pigment is a protective screen for chlorophyll, absorbing the short wavelengths of light; I have shown (2) that this complex appears as a storage substance. Since lipid alone can fulfill this function, what is the role of the astaxanthin? The frequent detection of carotenoids in the storage tissues leads us to think that these pigments must fulfill a general function and, due to their high capability of acting as a reducer, we are also led to think that they play a role in the preservation of the lipid or the storage substance with which they are associated. I have, therefore, tried to examine the antioxidant properties of astaxanthin in algae, the carotene in carrots and vitamin A.

The first of these pigments was extracted from *Trentepohlia* and then purified; the two remaining were provided to me, in their pure form, by industry. To characterize this function, I examined the most sensitive reagent: the biological reagent. It is known that mushrooms secrete oxido-reduction enzymes; the mushrooms oxidize the phenols, giving them color, while the oxido-reduction enzymes cause a more or less complete de-coloration of the methylene blue. *An antioxidant slows the oxidation reactions and, in contrast, accelerates the reduction reactions*. Having studied several different molds [fungi], I was able to determine that *Mucor Rouxii Welm* secretes oxidases when they are cultivated in the following milieu, adjusted to a pH of 8.1: water 1000, glucose 30^g, $PO_4(NH_4)$ 2H 1^g, NO_3NH_4, 1^g, SO_4K_2 0^g,40,SO_4Mg

(1) Meeting on June 24, 1946.
(2) Minutes, 221, 1945, pp. 707-708.

$7H_2O$ 0.20g, $SO_4Fe.SO_4$ (NH_4) 2^g, $6H_2O$ 0.10g, $SO_4Zn.7H_2O$ 0.04g, $SO_4Mn.7H_2O$ 0.05g, $SO_4Cu.5H_2O$ 0.02g, agar 25g.

I added a drop of guaiacol solution, 1% per 5 ml of milieu, which is then cooled in an inclined position. The difficulty comes from the fact that carotenoids are not easily soluble except in lipids: I added the oily solution just before the solidification of the milieu and I energetically add it in order to emulsify the mix. The control tubes must be shaken in the same manner because this operation introduces air into the mass and it is necessary to operate under identical conditions (especially with regards to the oxygen).

I prepared an equal number of control tubes and tubes containing 1/2500 carotene or 1/2500 astaxanthin or 1 drop of a vitamin A solution at 1/120,000 per gram.
These tubes were divided into 2 identical series, one placed in the light and the other in darkness, while the temperature is kept at 25° in both cases.

At the end of 3 to 4 days, the agar in the control tubes turned red, in darkness as well as in the light. In the following days, the color increased then slowly diminished (perhaps a little more quickly in the tubes exposed to light). During this time, the tubes containing carotenoids remained colorless or, sometimes very lightly colored pink but this light coloration is slower and disappears very quickly. I believe that it is produced in tubes in which the emulsification of the oily substance is less complete. The substances studied completely prevent oxidizing catalysis caused by mushrooms or, make it more or less minimal and transitory.

Although, among the species used, *M. Rouxii* alone secretes oxidases in a regular and consistent manner, it is not the same with reduction enzymes: all of the stem cells produce it in abundance. I, therefore, examined *Mucor mucedo L.*, which develops quickly and rapidly. Mushrooms are cultivated in an agarized Sabouraud milieu adjusted to a pH of 8.

I added a drop of 0.50% methylene blue solution per milieu tube which was then cooled in a vertical position; the mold was then well-sown into it. Carotenoids were used, at the dosages indicated in the first test and added by taking identical precautions. Two parallel series were prepared, one was placed in the light and the other in the darkness.

After 24 hours of culture, the tubes containing the vitamin began to lose color in the lower part (in the light and in the dark).

At the end of 36 hours of culture, I was able to create the following table:

Control	Carotene	Astaxanthin	Vitamin A
Light			
The tubes have not moved.	Tubes are discolored in the upper ½, green in the lower ½.	The tubes were discolored in the lower ¾ and green in the upper ¼.	The tubes were discolored almost completely in the upper portion.
Darkness			
Identical	Identical	Slightly more discoloration than in the identical tubes placed in the light	Identical

Meeting on July 1, 1946

After 2 days of culture, the controls began to become discolored in the lower parts. In general, the tubes developed quickly to a more or less advanced reduction stage and, later, the coloration did not vary much.

At the end of 10 culture days, the situation was as follows:

Control	Carotene	Astaxanthin	Vitamin A
Light			
The tubes turned blue on the surface, +/-clear green in the rest of the tube.	Tubes are discolored in the upper ½, clear green in the lower ½.	Most of the tubes were completely discolored, some slightly green at the surface.	All of the tubes were completely discolored.
Darkness			
Identical	Identical	Many of the tubes were completely discolored, the others were almost entirely discolored; they only had a few small patches of clear green.	Identical

The antioxidant properties of the carotenoids which were studied were associated with a more rapid and complete discoloration (never observed in the control tubes).

Astaxanthin and vitamin A are powerful anti-oxidants; the carotene used was a little less active. The action of the two latter is identical in the light and in the darkness; the former seems to be a little higher in the light: under natural conditions, it is the only one of the 3 substances studied which is located in cells exposed to the light.

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Date article was emailed to us	*Author, Title and Journal*	*Our file name*
June 6, 2013	Title: Antioxidant properties of carotenoids and their derivatives. Author: Armand HERISSET Journal: Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, v.253, pp. 47-49 (July – December) 1946.	*Herisset C.R.Ac.Sc. v223 p.47 Antiox_Eng Final*

6/12/2013
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Jun 12 2013
Notary Public Date

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