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Stability of Copper and Magnesium Chlorophylls

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ABSTRACT

The comparative stability of MgChl and CuChl to light, oxygen, temperature, sodium bisulfite, benzoyl peroxide and acid was studied by measuring the decrease of the absorbances at 650 and 660 nm of CuChl and MgChl solutions in n-*propanol, respectively.*

Values for the apparent first order reaction rate constant and for the half-life time were calculated. Results indicated that, except for benzoyl peroxide which rapidly decolorizes both pigments, CuChl is considerably more stable than MgChl following the increased covalent character of the bond between ligands and the d orbitals of Cu^{2+}.

INTRODUCTION

The substitution of synthetic pigments used in foods by the natural ones would be an important step to safer processed foods (Francis, 1987), but the absence of sufficient information on the properties of many natural pigments is one serious obstacle to their use.

Among the natural colours both CuChl and MgChl are two of the porphyrin pigments permitted in the EEC Directive of the 23-10-62 under the EEC N, E141 and E140, respectively (Coulson, 1980). The extraction of MgChl and the preparation of the corresponding Cu derivative have been described by Aronof (1953), and CuChl properties have been briefly reviewed by Humphrey (1980).

This paper reports the results of a study on the effects of light, oxygen, acid, sodium bisulfite, benzoyl peroxide and temperature on model systems of CuChl and MgChl.

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MATERIALS AND METHODS

Copper chlorophyll was prepared from extracts of the dried leaves of *Stevia rebaudiana* following the method described by Aronof (1953). The crude waxy product dissolved in chloroform was washed exhaustively with distilled water and elimination of the chloroform by distillation under reduced pressure produced a black waxy residue that was shaken with *n*-pentane and filtered. A solid black powdery product was obtained which was dried under vacuum and stored at $-15°C$ under N_2. TLC of the purified CuChl was done on silica gel plates developed with benzene:hexane:*n*-propanol (15:3:2).

The estimation of free copper in the purified CuChl was carried out according to the method recommended by FAO/WHO (1970).

Magnesium chlorophyll was prepared from fresh spinach leaves extracted with acetone with the addition of $CaCO_3$. The crude product was purified by preparative TLC on silica gel using hexane:benzene:ethanol (100:20:7) as the developing solvent.

Model systems consisted of solutions of CuChl (1·02 g/litre) and MgChl (0·36 g/litre) in peroxide-free *n*-propanol, distributed into 10 ml screw cap tubes. Irradiation was provided by a 40 W 'day light' lamp, with a nominal luminosity of 2500 Lm kept at a distance of 5 cm from the pigment

TABLE 1

Per cent of Colour Losses of Cu and Mg Chlorophylls, Reaction Times, up to 500 h, and Values for k (h^{-1}) and $t_{1/2}$ (h) at 25° ± 0·5°C

Reaction conditions in propanol	*% of colour losses*		$k \times 10^{-4}$		$t_{1/2}$	
	MgChl	*CuChl*	*MgChl*	*CuChl*	*MgChl*	*CuChl*
O_2/dark	7·0	0·7	0·79	0·23	0·88	3·00
O_2/light	95·0*	42·0	$66·4 \times 10^4$	3·45	$0·01 \times 10^{-4}$	0·20
O_2/dark/H^+	45·0	1·8	55·7	0·23	0·05	3·00
O_2/light/H^+	96·7**	43·7	$67·0 \times 10^4$	10·6	$0·01 \times 10^{-4}$	0·06
N_2/dark	6·0	0·5	0·69	—	1·00	—
N_2/light	91·0**	42·0	$98·6 \times 10^4$	8·75	$0·007 \times 10^{-4}$	0·08
$Na_2S_2O_5{}^{100}_{200}$ ppm/dark/N_2	0·0	0·0	—	—	—	—
BzO_2/dark/O_2/500 ppm	100·0****	100·0***	—	—	—	—
O_2/dark/40° ± 0·5°C	16·0	2·0	2·76	0·23	0·25	3·00
O_2/dark/60° ± 0·5°C	36·0	16·0	8·25	3·22	0·08	0·22

**** less than 5 h.

*** 24 h.

** 25 h.

* 45 h.

containing tubes. A N_2 atmosphere was made by thoroughly flushing each tube headspace with N_2.

Reaction conditions together with the calculated values for the apparent first order reaction rate (k) and the half-life time ($t_{1/2}$) are presented in Table 1. The loss of pigment in each system was followed by measuring the absorbance of the solutions at 650 nm for CuChl and 660 nm for MgChl using a Pye Unicam SP 8000 Spectrophotometer.

RESULTS AND DISCUSSION

Although a satisfactory yield (5·7%) of CuChl could be obtained from the chloroform extracts of *S. rebaudiana* the presence of considerable amounts of pheophytin in the extracts prevented its use for the preparation of MgChl.

The amount of free copper in the CuChl was found to be less than 200 mg/kg which is below the minimum required by FAO/WHO (1970) of 200 mg/kg. The decrease in the absorption values (%) for each system was taken as a measure of the pigment losses in each system.

Results in Table 1 indicate that, while in the CuChl solutions only 42·0% of the pigment was lost after 500 h, the loss of MgChl reached 95·0% after only 45 h of exposure to light. Both pigment solutions are completely decolorized by benzoyl peroxide in less than 24 h. Bisulfite, frequently present in processed foods, did not affect the CuChl or MgChl, and therefore did not produce alterations in the absorbance values of the pigment solutions.

The decreases in the absorbance of pigments heated to 40° and 60°C are possibly due to allomerization of the molecules as observed earlier by Jones *et al.* (1968). Allomerization could also be responsible for the loss of CuChl in the presence of acid. Absorption spectra for the system CuChl-H^+-O_2 did not show alterations at 647 nm, excluding pheophytin formation which occurs readily with MgChl. Substitution of Mg^{2+} for H^+ is easily achieved in MgChl but very drastic destructive conditions are needed to eliminate the Cu^{2+} from CuChl (Humphrey, 1980). This implies a stronger bond between the pyrrolic nitrogen ligand and the Cu^{2+}. The presence of 3d orbitals in the Cu^{2+} will increase the covalent character of the bond between ligand and the Cu^{2+} acceptor making the structure of the CuChl more stable (Falk, 1964).

Although the results in Table 1 indicate that CuChl is a more stable pigment, a higher concentration of CuChl (1·02 g/l) was needed to achieve an absorbance similar to that obtained with a concentration of 0·36 g/litre of MgChl.

CONCLUSION

Copper chlorophyll is a stable fat-soluble pigment which can be easily obtained from abundant and cheap by-products. Its stability makes it a good substitute for Mg-chlorophylls and synthetic colorants when water solubility is not of prime importance; its apparent tinctorial capacity is roughly one third that of pure Mg-chlorophylls.

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