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A SIMPLE TECHNIQUE FOR INDUSTRIAL ANALYSIS OF TOTAL CHLOROPHYLL

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The analysis of chlorophyll has been well discussed by many authors, including MCKINNEY (*3*), ZSCHEILE (*4, 5*) and the Merck team (*2*). However, an easy technique that combines speed and simplicity with enough precision for industrial purposes is needed for use in the purchase of raw material for production of this pigment and its derivatives.

Materials and methods. Lots of 100 g of green leaves of the mango tree (*Mangifera indica* L.) were dried at 105°C for three hours, finely ground, sifted through a Tyler 200 sieve and kept in an amber glass desiccator. Sets of ten 500 mg samples were taken into 60 ml stoppered vials, and 30 ml of 80% (v/v) acetone was added to each vial. The stoppered vials were left in the dark for periods of two, three, four, five, seven and eight hours.

At the end of each time period each sample was filtered through dry paper (S & S No. 589 white band) into a 25 ml volumetric flask and the volume completed with the acetone solvent. The extraction was done in subdued light with a bulb mounted 3 m above the work surface, giving 25 lumens over an area of 15 m^2. The clear solutions were diluted 1:10 with the solvent, samples placed in square quartz cuvetes and the absorbance measured at 663, 645, 660 and 642.5 nm with a Perkin-Elmer-Coleman No. 124-DB spectrophotometer.

Total chlorophyll content was calculated according to the following formulas:
Formula a (*4*): Total chlorophyll = $(8.04 \times E_{663} + 20.441 \times E_{645})$ mg/l.
Formula b: Total chlorophyll = $(9.68 \times E_{660} + 22.84 \times E_{642.5})$ mg/l.
For comparison, total chlorophyll was also determined by the photoelectric colorimetric method of the Association of Official Agricultural Chemists (A.O.A.C.) (*1*).

Results and discussion. The results are given in Tables 1 and 2. Analysis of variance yielded a value for F that was significant at the 1% level, and the coefficients of variation for formulas a and b of 7.75 and 6.79, respectively. According to the Tukey test, there was no significant difference at the 1% level between methods from three to eight hours extraction time, but the difference was significant for two hours extraction time.

The relatively high coefficients of variation could be explained by the variances for treatments of two and eight hours. In the first case, there was probably poor extraction because of lack of time for a more intimate contact between solvent and sample; and in the latter case, it is possible that some decomposition of the pigment had taken place.

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TABLE 1 - Efficiency of extraction in mg/1 as related to time in hours, calculated by formula a

Sample	Time 2 hours	3 hours	4 hours	5 hours	7 hours	8 hours	A.O.A.C.
1	2.16	2.99	3.39	3.23	2.71	3.35	3.38
2	2.13	2.88	3.20	3.24	2.81	3.32	3.25
3	2.22	2.84	3.08	3.19	2.78	2.86	2.98
4	2.74	2.68	3.25	3.13	2.83	3.47	3.04
5	2.72	2.51	3.21	3.09	2.84	2.73	2.70
6	2.24	2.84	3.16	3.04	3.09	2.66	2.83
7	2.56	3.45	3.10	3.08	3.08	2.66	2.87
8	2.87	2.84	3.09	3.08	3.08	3.48	3.03
9	2.77	2.73	3.21	3.56	3.08	3.08	2.75
10	2.77	2.79	3.09	3.13	3.08	2.70	2.85
εx	25.18	28.55	31.78	31.77	29.38	30.31	29.68
$\bar{X}$	2.52	2.86	3.18	3.18	2.94	3.03	2.97
S^2	0.0875	0.0603	0.0094	0.0225	0.0242	0.1207	0.0467

For a relatively simple technique, the authors suggest the use of formula b with absorbances measured at 660 and 642.5 nm in 10 mm cuvetes, after an extraction time of four hours. This procedure saves eight hours when compared with the usual industrial procedures, which require 12 hours, and provides an analysis that does not require much time from the technician, allowing him a free hands period of four hours.

RESUMO

Os autores desenvolveram uma técnica, para análise espectrofotométrica de clorofila total, simples, rápida e bastante precisa para uso pela indústria quando da compra de matéria-prima para produção deste pigmento e seus derivados.

A técnica proposta apresenta uma economia mínima de 8 horas de trabalho analítico quando comparada com métodos tradicionais, com resultados não diferentes estatisticamente destes, e se baseia na extração a frio do pigmento com acetona 80% (v/v), durante quatro horas, seguida de espectrofotometria nos comprimentos de onda 660 e 642.5 nm.

TABLE 2 - Efficiency of extraction in mg/l as related to time in hours, calculated by formula b

Sample	Time 2 hours	3 hours	4 hours	5 hours	7 hours	8 hours	A.O.A.C.
1	2.24	2.91	3.34	3.23	2.81	3.47	3.38
2	2.24	2.91	3.23	3.34	2.84	3.34	3.25
3	2.45	2.91	3.17	3.26	2.79	2.92	2.98
4	2.81	2.84	3.26	3.21	2.81	3.34	3.04
5	2.81	2.50	3.38	3.26	2.88	2.73	2.70
6	2.31	2.87	3.23	3.11	3.17	2.79	2.83
7	2.64	2.84	3.19	3.17	3.15	2.67	2.87
8	2.79	2.87	3.21	3.19	3.15	3.23	3.03
9	2.79	2.84	3.26	3.46	3.15	2.75	2.75
10	2.85	2.86	3.17	3.23	3.19	2.75	2.85
εx	25.93	28.35	32.44	32.46	29.94	29.99	29.68
$\bar{X}$	2.59	2.84	3.24	3.25	2.99	3.00	2.97
S^2	0.065	0.0147	0.0048	0.0093	0.0325	0.0958	0.0467

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