

Simple Colorimetric Measurement of Citral in Lemon Scented Essential Oils Using Schiff's Reagent

Nativ Dudai, Olga Larkov and Efraim Lewinsohn
Division of Aromatic Plants, ARO, Neve Ya'ar Research Center
P.O.B. 1021 Ramat Yishay 30095 Israel

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Abstract

Citral is a key component of lemon scented essential oils. In our aromatic plant breeding programs it is necessary to rapidly screen hundreds of samples for their citral content. The current method based on GLC analysis is expensive and time consuming. Here, we describe a colorimetric method for citral determination based on the Schiff's reaction of citral. The method is rapid, reproducible and simple, but must be calibrated against GLC. The method is applicable only to samples in which citral is the preponderant reacting aldehyde present.

INTRODUCTION

Citral is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B). Usually one isomer does not occur without the other in nature. Citral is a chief constituent of essential oils of many lemon-scented aromatic plants. Because of its characteristic lemon aroma, citral is of considerable importance in the food and flavour industry. Citral is also an important raw material used in the pharmaceutical, perfumery and cosmetic industries, especially for the synthesis of vitamin A and ionones; synthetic citral derived from conifer turpentine is normally used for those purposes (Dawson, 1994). Citral possesses antifungal activity against plant and human pathogens (Yousef et al., 1978; Rodov et al., 1995), inhibits seed germination (Dudai et al., 1999), and has bactericidal (Asthana et al., 1992; Kim et al., 1995) and insecticidal properties (Rice and Coats, 1994).

The Schiff reagent interacts with many aldehydes to obtain an intense color and it is used extensively in light microscopy to stain cell walls. In a previous study, we used the Schiff's reagent in combination with light microscopy, to stain single oil accumulating cells in the leaf mesophyll of lemongrass (Lewinsohn et al., 1998). In the present study we describe the development of a colorimetric method for the analysis of citral using Schiff's reagent. The use of Schiff's reagent to assay citral has been described in the past (Snell and Snell, 1953). Utilizing this method, many lemon-scented botanical samples can be tested simultaneously, by using very simple equipment, facilitating the screening of a large number of samples in a short time. We routinely assay hundreds samples a day.

MATERIALS AND METHODS

Schiff's reagent (1 % pararosaniline chloride and 4 % sodium bisulphite in 0.25 N) was from Sigma. As a standard we used analytical citral (98 % Sigma). The absorption spectrum of the reaction product between Schiff's reagent and citral was measured in the range of wavelength of 400-700 nm. For routine assays, the absorbance at 580 nm was used.

To quantify the amount of citral in essential oils, calibration curves were first constructed. The reaction was performed in 1.5 ml polypropylene tubes at room temperature. The assay system contained 800 μ l of water, 100 μ l of Schiff's reagent and 100 μ l of citral solution in ethanol with 0 to 1000 nmol of citral. The calibration was made in a range of 0 to 1000 nmol of citral. After various periods of incubation, A₅₈₀ was measured.

Essential oils were hydrodistilled for 1.5 h from 250 g of shoots of fresh aromatic plants harvested from experimental plots of the Neve Ya'ar Research Center, Israel, using a modified Clevenger apparatus (Ravid et al., 1997). The composition of the essential oils

in the distillates was identified using gas chromatography and GC-MS analysis described previously by Ravid et al. (1997).

RESULTS AND DISCUSSION

Development of the Test Method

1. Absorption of the Reaction Product Between Citral and Schiff's Reagent. The Schiff's reagent gives a red color as a result of the reaction with some aldehydes, including citral (Snell and Snell, 1953). The absorption spectrum of the reaction products is given in Fig. 1, with the maximum at 580 nm.

2. Dependence of Color Intensity on Length of Incubation and on Citral Concentration. To assess linearity of the method and find optimal conditions for its use, we followed the development of color over 72 h (Fig. 2). The absorption at 580 nm increased rapidly within the range of 100 to 1000 nmol citral. At higher citral concentrations absorbance at 580 nm reached a plateau after 24h while at low concentrations it continued to increase up to 72 h. Fig. 2 shows a useful relation between O.D. at 580 nm and citral concentration up to 1000 nmol following 1 h. incubation, up to 600 nmol following 5 to 30 h, and up to 400 nmol following 72h.

3. Calibration Curves - Response of the System to Various Amounts of Citral. To quantify the amount of citral in essential oils, calibration curves against GLC measurements were made. The calibration was made in a range of 0 to 1000 nmol of citral. After various periods of incubation, A₅₈₀ was measured. A linear response was possible to achieve after only 1 hour, but the maximal response to small amounts was obtained only after a longer incubation time (72 h) (Fig. 3). At low levels (200 to 400 nmol) of citral the system stabilized after 24 h (Fig 4). From these results we concluded that a calibration curve should be produced with every run to keep accuracy of the determination.

4. The Effect of Temperature. The reaction of citral with Schiff's reagent was sensitive to the temperature of the incubation. Increase in temperature increased the rate of the reaction, as expected. However, in the range of 22 to 27°C only minor differences in the reaction rates were noted. The temperature effect was observed already after one hour of incubation (Fig. 5a) as well as after 24 hours (Fig. 5b). The optimal level appears to be 25 ± 2°C.

Measurement of the Citral Content in Various Essential Oils

To calibrate the method to measure the citral content in lemon scented essential oils, we compared the results obtained with the Schiff reagent with those obtained by GLC conventional measurements. A high correlation was found between the results obtained by each the two methods (Fig. 6). However, although sweet basil does not contain any citral, low absorbtion values were observed in part of the replicates (Fig. 6).

The results indicate that this is a simple and effective method for measuring the citral content in some essential oils without the need for expensive equipment (such a GLC) or complicated laboratory instruments. This method is limited to essential oils known to contain citral as the only (or preponderant) colour reacting aldehyde. The method requires calibration by using pure citral. This method is rapid and allows the examination of many samples simultaneously. GLC analysis takes up to 1 hour per sample.

The citral concentration is important for characterization of the essential oils present in lemon grass (*Cymbopogone citratus*) or lemon balm (*Melissa officinalis*), since it is a factor in the quality of the fragrance. This simple and rapid method of analyzing many samples may be of help, for example in primary genetic selection isolate high citral clones in variable populations.

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Figures

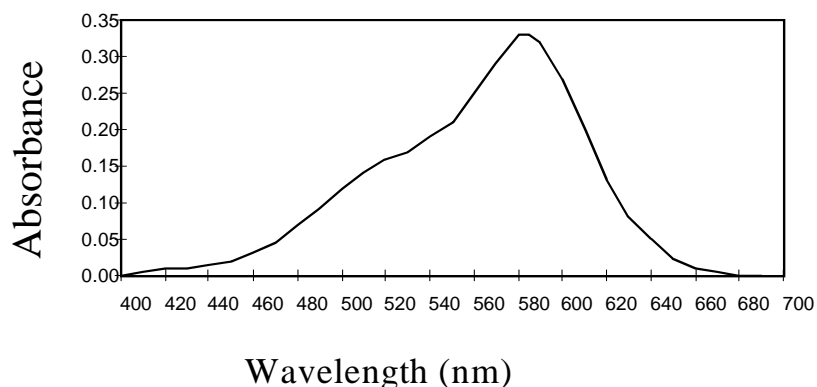


Fig. 1. Absorption spectrum of reaction product of citral with Schiff's reagent.

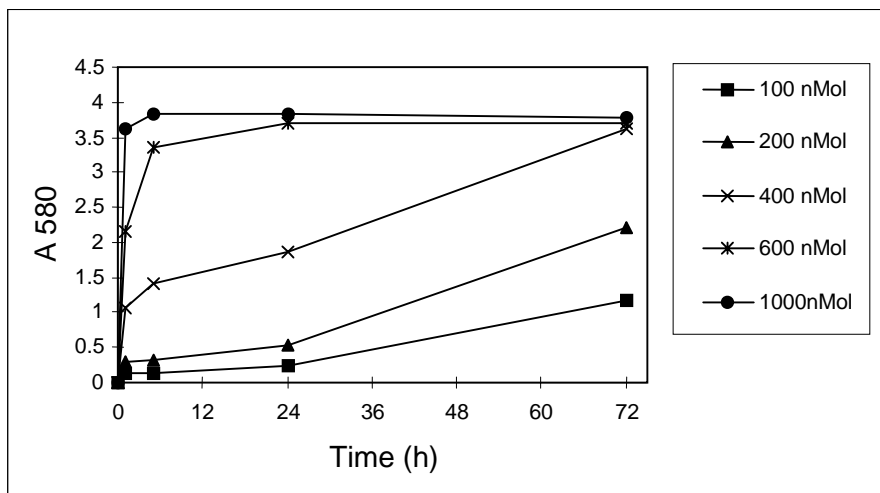


Fig. 2. Change in the absorbance at 580 nm as a function of time at various citral concentrations.

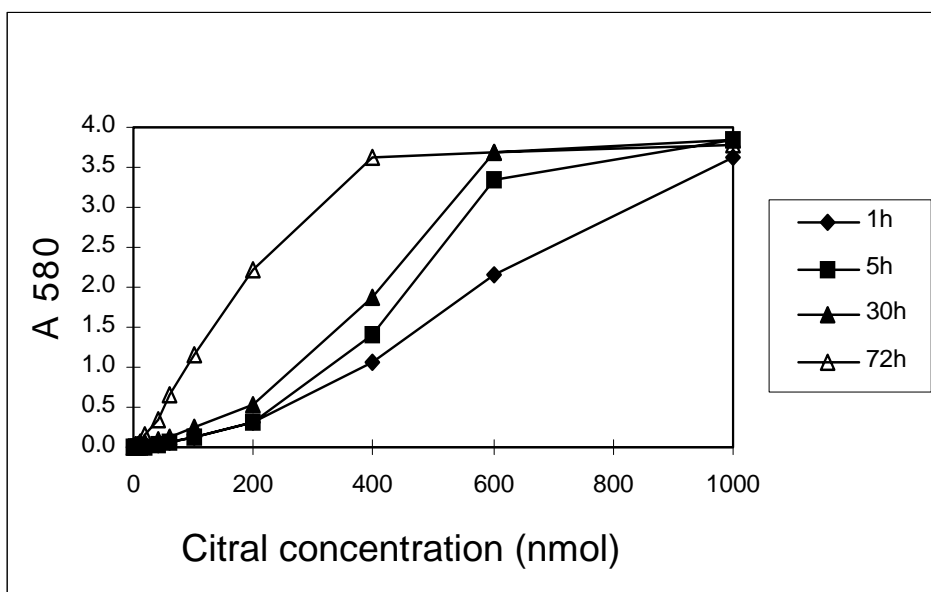


Fig. 3. Effect of citral concentrations on the absorbance at 580 nm following different incubation times with Schiff's reagent.

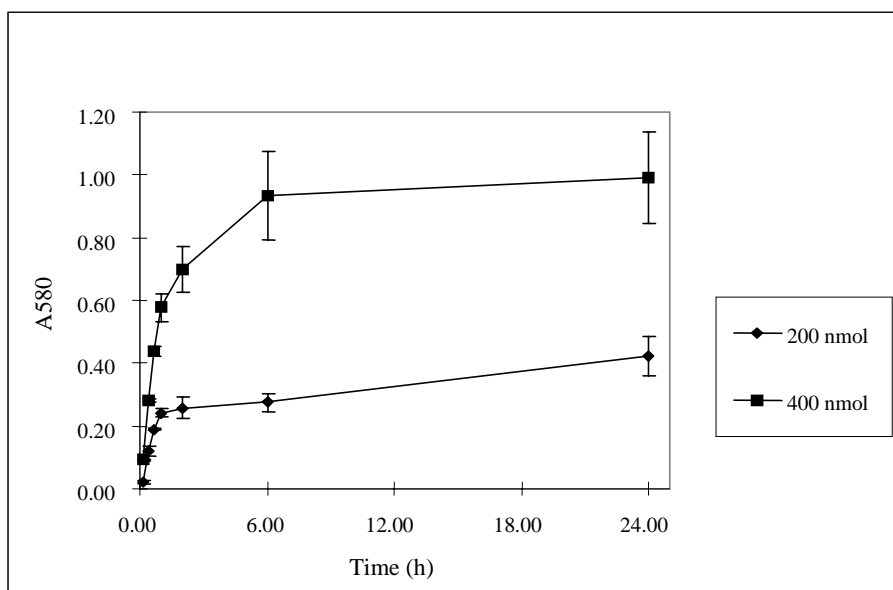


Fig. 4. Development of absorbance at 580 nm with time at 2 citral concentrations.

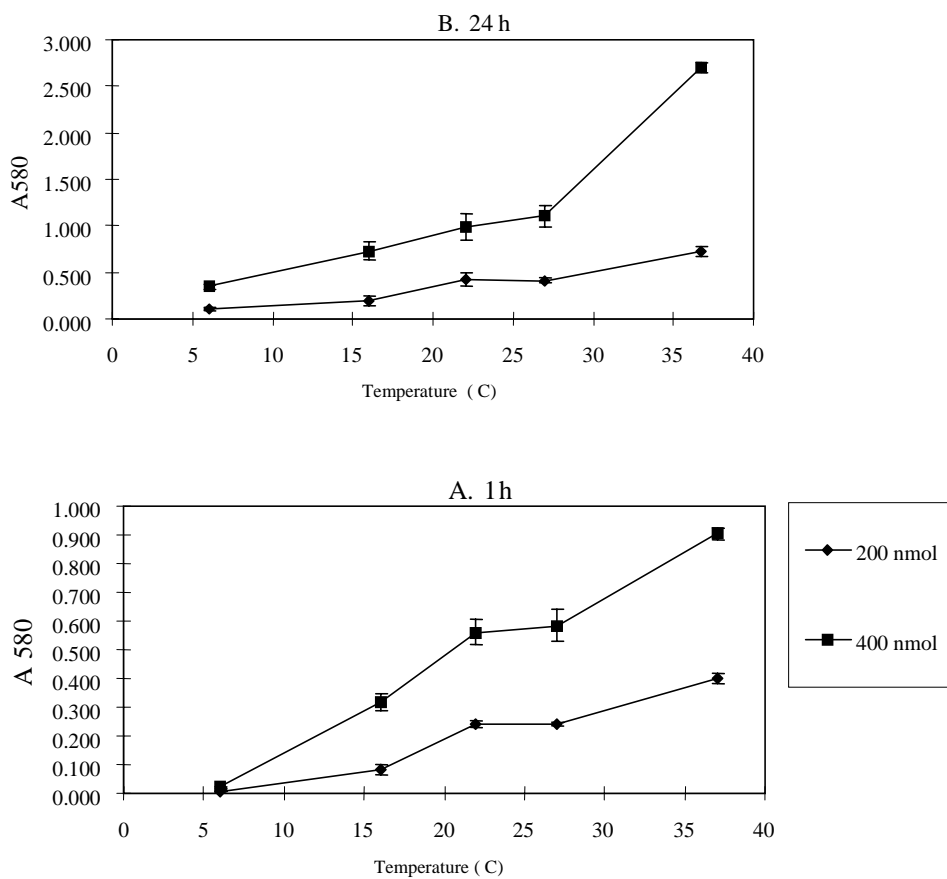


Fig. 5. The effect of temperature on the absorbance at 580 nm after one hour (A) and after 24 hours (B) of incubation.

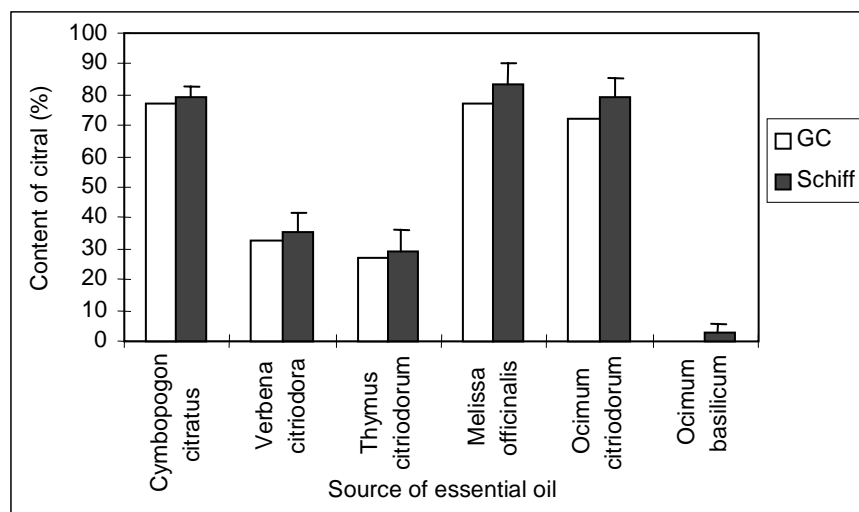


Fig. 6. Comparison between GLC and Schiff's reagent determination of citral content of different essential oils.