Fermentation Inhibition by 2,6-Dichloro-4-nitroaniline (DCNA)

The effect of DCNA (2,6-dichloro-4-nitroaniline) on the fermentation rate of peach wine produced in Georgia was determined. DCNA was identified by GC–MS and quantitated (1.2 mg/l.) by GLC in peach concentrates that fermented more slowly than normal. The effect of the DCNA on the growth of a yeast test strain (*Saccharomyces cerevisiae var. ellipsoideus*) was studied in basal salts media containing sucrose (50 g/l.) and in basal salts media containing ethanol (8% v/v) and sucrose (2.50 g/l.). At DCNA concentrations similar to or higher than those in the concentrate, cell growth was not affected in the nonalcoholic media. However, at 0.25 mg/l. DCNA in the alcoholic media (made to simulate wine vat conditions), the lag phase time for the yeast was increased 8 h and the total cell density was decreased by 50%.

Native fruits, such as peaches and grapes, are used in the production of wine in Georgia. To maintain continuous production of peach wine throughout the year, a peach concentrate is made by vacuum evaporation during the peach season. In 1969, however, some local wineries began to experience low fermentation rates with some batches of peach concentrate. For some batches, the fermentation time to reach a given alcohol content was 3 to 4 times the normal fermentation time. In some cases, only 8% alcohol was achieved after 3 months fermentation in a wine fermentation vat (Veit, 1974). The decrease in fermentation rate therefore decreased the value of the peaches for wine production. The present study was undertaken to determine the cause of the erratic fermentation rates.

Kilgore et al. (1962) reported the presence of the fungicide 2,6-dichloro-4-nitroaniline (DCNA; dichloran; Batian; produced by the Upjohn Co.) in processed peach concentrate. Since DCNA is recommended and is widely used in the Southern states on stone fruits, such as peaches, to control harvest fungus rots, the possible role of DCNA in the decreased fermentation rate was investigated (Ogawa et al., 1961; Harris and McGlohon 1974).

EXPERIMENTAL SECTION

Procedures. Quantitative analysis for DCNA was achieved by extraction of 1 ml of peach concentrate with 10 ml of isooctane. DCNA concentration was then determined by GLC analysis of the extract and comparison of peak heights with those of standard solutions.

For mass spectral studies, 1.5 l. of the peach concentrate was extracted with 100 ml of isooctane. The isooctane layer was removed and concentrated to 0.5 ml on a rotary evaporator.

Growth media for testing were prepared by adding DCNA dissolved in acetone to basal salts solution containing sucrose at two different concentrations (50 g/l. and 2.5 g/l.). The two sugar concentrations were tested since sugar concentration can affect the toxicity of some pesticides (Ashton et al., 1966). Six different DCNA concentrations were tested at each sucrose level: 0.1, 0.25, 0.50, 0.75, 1.0, and 3.0 ppm of DCNA.

The above experiments were repeated, at both sucrose concentrations, in media containing 8% ethanol. The medium containing alcohol and 2.5 g/l. sucrose most accurately duplicates the wine vat conditions under which the decreased fermentation efficiency was observed.

The test strain of yeast was grown in 200 ml of nutrient broth in 500-ml Erlenmeyer flasks on a rotary shaker before inoculating it into the test media.

To monitor the growth curves, 100 ml of each test medium was placed in 250-ml side-arm flasks inoculated with 1 ml of inoculum and incubated at 30 °C in light. The optical density (OD) of the growing culture was read at approximately 1-h intervals with more frequent readings during periods of active changes in the growth patterns.

Apparatus. The gas–liquid chromatography (GLC) studies were carried out on a Tracor MT-200 chromatograph equipped with a ^{63}Ni electron capture (EC) detector. Retention times were compared using a 2 ft × 0.25 in. glass column containing Chromosorb W coated with 3% methylsilicone (SE-30); the inlet, column, and detector temperatures were 200, 150, and 250 °C, respectively.

GC–MS analyses were performed with a Varian Aerograph Model 1532-B gas–liquid chromatograph and a Finnigan 1015 SL quadrupole mass spectrometer having a gas jet separator and a Systems Industries 150 digital computer. A 6 ft × 0.25 in. glass column containing Chromosorb W coated with 3% methylsilicone (SE-30) was employed. Column conditions were as follows: inlet temperature, 250 °C; column temperature programmed from 100 to 250 °C at 10 °C per min.

The growth curves were monitored on a Bausch and Lomb Spectronic 20 colorimeter, read at 570 m.

Samples and Reagents. One-gallon samples of each fruit concentrate to be tested were furnished by Monarch Wine Co., Atlanta, Ga. Each sample was taken from a batch of fruit concentrate with a known fermentation history. The test strain of yeast was *Saccharomyces cerevisiae var. ellipsoideus*, originally obtained from C. D. Webb, mycology proficiency testing section of C.D.C., Atlanta, Ga.

The 2,6-dichloro-4-nitroaniline (DCNA) was obtained from Chem Service and was used as received. Isooctane was purchased from Burdick and Jackson.

RESULTS AND DISCUSSION

Batches of peach concentrate that had shown low fermentation rates in the winery were analyzed for possible yeast inhibiting compounds that were not present in concentrates that fermented normally. Analysis of the peach concentrate extract was first performed by GC–MS. Figure 1 shows the reconstructed gas chromatogram from one batch. Based on mass spectrum 127 of this chromatogram (Figure 2), the first significant peak was identified as 2,6-dichloro-4-nitroaniline (DCNA). This spectrum was identical with that of an authentic sample of DCNA and with the DCNA mass spectrum reported by Hutzinger et al. (1971). GLC analyses of the isooctane extract employing an electron capture (EC) detector gave a single chromatographic peak with a retention time identical with that of an authentic DCNA sample.

Based on mass spectral data, the remaining peaks in the chromatogram were determined to be phthalates, hydrocarbons, and fatty acid esters.

The DCNA was found to be present at a concentration of 1.20 ppm in the slow-fermenting peach concentrate and

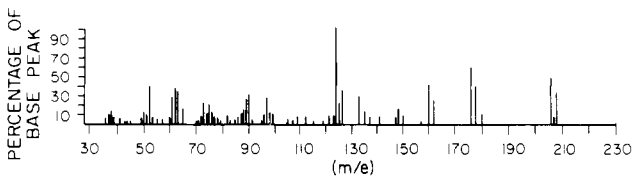

Figure 1. Reconstructed gas chromatogram of peach-isooctane extract.

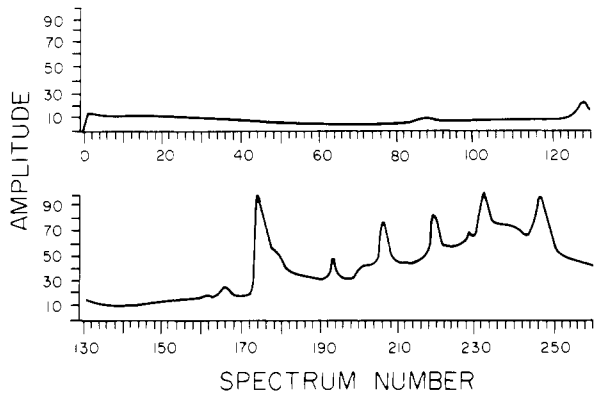

Figure 2. Mass spectrum no. 127 with spectrum no. 129 subtracted as background.

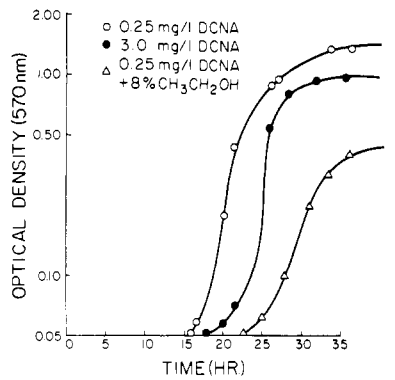

Figure 3. Growth curves at various concentrations of DCNA.

was not present in the batches that fermented normally.

DCNA might be expected to be present in the peach concentrates since the fungicide is recommended to be used on the tree ripening fruit up to the day of harvest and on the harvested fruit at the hydrocooler. However, the recommended concentration for the spray is only about 900 ppm in a water suspension (Veit, 1974). With such low concentrations, it is not likely that a relatively high concentration of DCNA would be found in the concentrate.

When the peach concentrate is reconstituted for fermentation at the winery, however, a constant dilution factor is not used. From 4 to 10 parts of water may be added to the concentrate, depending on its pH. This factor may give rise to the erratic nature of the problem.

The slow-fermenting concentrate examined in these laboratory studies contained 0.25 ppm of DCNA. In the nonalcoholic laboratory cultures containing 0.25 ppm of DCNA, however, no effect on the yeast growth curve was observed. In nonalcoholic media containing as high as 3.0 ppm of DCNA, although the total yeast cell concentrations were decreased, the lag phase time was increased by only 2 h.

However, in the media that were made to duplicate the wine vat conditions (8% ethanol and 2.50 g of sucrose/l.), a DCNA concentration of 0.25 ppm increased the lag phase time by 8 h and decreased the total cell density by more than 50% (Figure 3).

This work indicates that DCNA does pass through the current peach processing method in sufficient quantities to cause, in the presence of alcohol, a significant decrease in yeast growth rate as experienced by wineries.

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Mineral Contents of Some High Yielding Varieties of Bengal Gram (*Cicer arietinum*)

Seeds of seven high yielding varieties of Bengal gram, grown at the same locality with similar fertilizer applications and farm practices, were analyzed for copper, iron, calcium, magnesium, sodium, and potassium contents. A wide variation for these minerals was observed among different varieties.

It is now increasingly realized that pulses in addition to being an important source of protein are also a good source of some minerals (Goswami and Basu, 1938; Chowdhury and Basu, 1939). The work on evolution of new high yielding varieties of pulses has opened a new vista for study. Copious literature is available to indicate that with the change in genotype, significant changes occur in protein and amino acid contents of pulses (Esh et al., 1959, 1960;