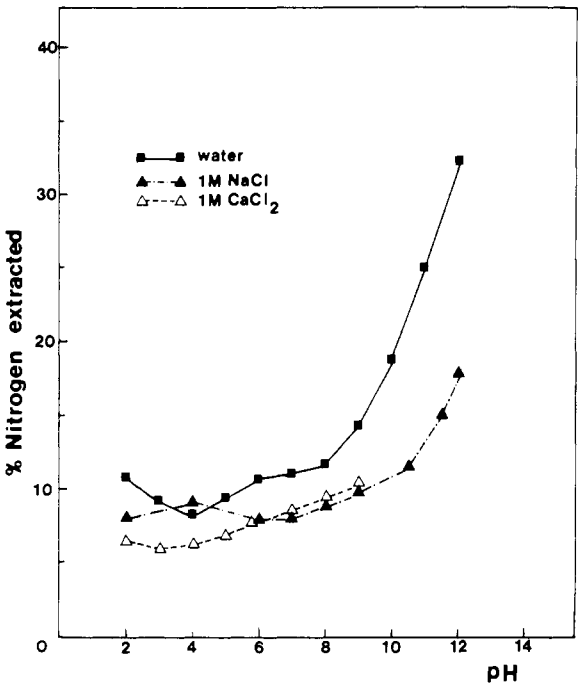


**Figure 1.** Solubility of the N of grape seed meal in water and salt solutions as a function of pH.

(Sodini and Canella, 1977). Further work is required to establish if a dehulled grape seed meal with a subsequently more soluble protein content and free of color-forming precursors would represent an interesting source of economic proteins to utilize in food preparation.

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# Biosynthesis of Flavor Compounds by Microorganisms. 3. Production of Monoterpenes by the Yeast *Kluyveromyces lactis*

The monoterpenes citronellol, linalool, and geraniol were found in the odorous constituents produced by the yeast *Kluyveromyces lactis* in aerobic submersed culture. Their biosynthesis did not require special precursors. Citronellol and linalool were produced at about 50 μg/L, while geraniol could be detected only in traces; added geraniol was reduced nearly quantitatively to citronellol. By changing the culture conditions it is possible to influence the yield of monoterpenes as demonstrated for citronellol. With increasing temperature and with increasing concentrations of asparagine, as a nitrogen source, citronellol was formed at a higher rate.

Monoterpenes are of interest because of their structural characteristics and their physiological and sensory activities. These properties result in broad applications in flavor industry, food industry, and pharmacy. In 1972 Devon and Scott listed about 400 monoterpenes, which are primarily products of higher plants. Little is known about the occurrence and biosynthesis of monoterpenes in microorganisms. In 1964 Katayama found geraniol, *d*-limonen, and α-pinen in algae. Collins and Halim (1970, 1972) and Halim and Collins (1971) reported the presence of monoterpenes in cultures of the fungi *Ceratocystis variospora*, *Trametes odorata*, and in various species of the fungus *Phellinus*. The mechanism of biosynthesis of monoterpenes in fungi was studied by Lanza and Palmer (1977) by feeding radioactive precursors to *Ceratocystis moniliformis*. As part of our studies on the biosynthesis of flavor compounds by microorganisms (Schreier et al., 1975, 1976) we found that the yeast *Kluyveromyces lactis* also produces monoterpenes.

EXPERIMENTAL SECTION

*Kluyveromyces lactis* No. 2359 was obtained from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. The organism, maintained on YM-agar (Difco), was cultivated in 1-L Pasteur flasks, plugged with cotton wool, containing 300 mL of modified (Difco) Yeast-Carbon-Base (C, N sources and ratios, vitamin supply). The inoculated media were shaken at 180 rpm, usually at 25 °C. After a cultivation period of 2 to 4 days the cells were removed by centrifugation. The clear culture broth was extracted with pentane–methylene chloride (2:1) in an apparatus for liquid–liquid extraction, free acids were removed with 5% $NaHCO_3$ solution, and the pentane–methylene chloride phase was then dried over anhydrous $Na_2SO_4$ and concentrated on a Vigreux column at 45 °C (Drawert et al., 1969). Gas chromatography was carried

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out on a Varian aerograph Series 1 800, with 5 m × 0.125 in. stainless steel column, packed with 5% FFAP on Varaport 80–100 mesh. The column, was programmed from 70 °C at 2 °C/min to 250 °C. For sensory tests of individual peaks, we used an equal size column, packed with 12% FFAP. The column outlet was split into two fractions: about 7% of the gas flow passed the flame ionization detector and the rest went to the sniffing tube. The temperature was programmed from 90 °C at 2 °C/min to 250 °C. Mass spectrometric analysis were performed after fractionation of the flavor concentrates on silica gel by means of column chromatography as described previously (Schreier and Drawert, 1974).

## RESULTS AND DISCUSSION

In the silica gel fractionated flavor extracts of *Kluyveromyces lactis* culture broths about 150 different compounds were detected by gas chromatography. Among the hitherto identified compounds are the monoterpenes citronellol, linalool, and geraniol which were identified with the aid of reference compounds. Samples, reference compounds, and samples with added references had identical properties in gas chromatography, in silica gel fractionation, in their sensory qualities (sniffing of single gas chromatographic peaks at the split outlet of the column), and in mass spectrometry. Citronellol and linalool accumulated at about 50 μg/L in the culture broth; geraniol, which is the first monoterpene formed via the mevalonic acid pathway (Banthorpe et al., 1972; Lanza and Palmer, 1977) could be detected only in traces. In experiments with added geraniol (0.05, 0.5, 5, and 15 mg/L) we found that geraniol is reduced nearly quantitatively (about 90%) to citronellol. Only 1–2% of the added geraniol remained unchanged after a culture period of 4 days.

Monoterpene biosynthesis by *Kluyveromyces lactis* does not depend on special precursors, but variations of culture conditions had an influence on their yield as we could show for citronellol. With increasing culture temperature (10, 15, 22, and 27 °C) citronellol was formed at a higher rate in a medium containing 20 g of glucose and 0.5 g of asparagine/liter, but no vitamins. These cultures were inoculated with washed yeast cells, grown for 30 h on an equal medium with vitamin supply. Relative yields under these conditions were 18, 22, 64, and 100%, respectively. The 100% value corresponds to 60 μg of citronellol/liter.

A similar effect was observed for increasing concentrations of asparagine (0.1, 0.2, 0.5, and 1 g/L) at a constant amount of 20 g of glucose/liter of culture medium. Citronellol yield rose with increasing asparagine concentration from 2% over 12% and 71% up to the final value 100%, respectively, 50 μg of citronellol/liter.

These results confirm previous findings that microorganisms are able to produce monoterpenes. By changing the culture conditions, it is possible to influence the biosynthesis of citronellol in the yeast *Kluyveromyces lactis*.

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# Effect of Heat Treatment on Amino Acid Composition of Canned Baby Food

The amino acid content of some canned baby foods was analyzed before and after normal processing including heat treatment at 70 °C for 1 h and at 121 °C for 50 or 60 min. In all heated products there was a decrease in total amino acids in proteins, but the percentage proportion of essential amino acids in the total amino acids remained unchanged. Available lysine content decreased by approximately 15% in all foods studied. The decrease was also found in chemical score values, which were about 10% lower after heat treatment. Only traces of lysinoalanine were found in some samples.

It is a well-known fact that when protein foods or isolated proteins are heated above 100 °C at about neutral pH, chemical changes take place and reduce the nutritive value of the proteins (Bjarnason and Carpenter, 1970; Osner and Johnson, 1974, 1975; Hurrell and Carpenter, 1976). Similar changes have also been found in canned baby foods during sterilization (Rakowska, 1972). One of the products of damaged amino acids in proteins is lysinoalanine, especially after heat treatment in alkaline conditions (De Groot and Slump, 1969; Woodard and

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