the Strecker degradation, *cf.* Baddar (1959). Realdolization of III with formaldehyde followed by dehydration of the intermediate carbinol IV would lead to a new imino ketone V which now contains a chain of four carbon atoms (*cf.* compound I with a chain of three carbon atoms). Hydrolysis of V could yield acetaldehyde plus a four-carbon aminoketone VI; and condensation of VI with aminoacetone with loss of water could ultimately yield trimethylpyrazine. Similarly, compound III may undergo realdolization with acetaldehyde (instead of formaldehyde), in which case a homologous aminoketone VII would be the product. Further condensation of VII with aminoacetone could ultimately lead to 2,5-dimethyl-3-ethylpyrazine as shown. Thus, the formation of trimethyl- and 2,5-dimethyl-3-ethylpyrazines can be explained in terms of a nonoxidative pathway involving a series of potentially reversible aldol condensations followed by thermal rearrangement of hydropyrazine intermediates to form aromatic products. Although highly speculative, the mechanism of Figure 2 does explain the formation of the wide variety of polyalkylpyrazines which have been reported to date. Extensions of the mechanism could involve participation of the various aldehydes formed during Strecker degradation of α-amino acids. Using this type of reasoning it is possible to predict the formation of many complex branched chain pyrazines, only a few of which have already been isolated from browned foodstuffs.

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Received for review December 6, 1971. Accepted January 28, 1972.

Extraction of Oil Samples from Seeds with Little Impairment of Viability

Seeds immersed in lipid solvents for several days release small amounts of their lipids without losing their viability. Thus, it is possible to determine the fatty acid composition of a seed sample and still be able to plant it and produce offspring.

The ability to determine the quantity and quality of oil in seeds prior to germination is of paramount importance both in basic genetic research involving parent–offspring comparisons and in plant breeding experiments. The traditional method of oil extraction for analytical purposes has consisted of expelling the oil by crushing the seed, followed by solvent extraction. Comparison of seed weight before and after oil removal determines the oil content of the seed, and a chemical analysis of the oil sample gives its composition. The problem with this technique, however, lies in the destruction of the seed, which the researcher may need for planting as parental material because of a desirable genetic constitution.

In recent years, wideline nmr spectroscopy has provided a quantitative means of rapid and accurate measurement of the lipid content of whole seeds without subjecting them to treatment which impairs their viability (Alexander *et al.*, 1967). In the absence of a better technique for oil quality determination, however, some investigators have cut off portions of a seed or removed one of its cotyledons for oil quality analysis and then planted the remainder of the seed to obtain progeny (Downey and Harvey, 1963).

The authors have now found an alternative, nondestructive method for oil extraction without impairment of seed viability. The technique consists of immersing seed samples for 4 to 6 days in lipid solvents at room temperature. The immersion process extracts amounts of oil from the seed which are sufficient for chemical analysis after evaporation of the solvent.

The technique has been employed with seed of safflower, flax, sunflower, soybean, and sesame and has proven satisfactory with samples as large as 50 g of seed in 500 ml of solvent and as small as a single seed in a 15 $\times$ 125 mm test tube with 5 ml of solvent. Since several drops of oil can be obtained from large seed samples, any of the conventional analytical methods may be used. Sufficient oil can be extracted from single seeds for glc analysis. Samples of 50 to 100 seeds from those plants mentioned above provide 15 to 25 mg of oil, sufficient for obtaining a reading on a refractometer. After termination of the immersion, seeds are air dried under a hood, and they may then be planted. Plants

obtained in our studies have grown normally to full maturity.

The reliability of the method has been verified by fatty acid analyses of oils obtained by the following three techniques from genetically homozygous seed samples: pressing untreated seed; extracting oil by immersion of seed in solvent; and pressing seed previously extracted by solvent immersion. Analyses of oil samples obtained by these three methods by glc provided essentially the same results for each seed sample. The glc analytical methods used were described in earlier reports (Yermanos *et al.*, 1972).

Solvents which have been tried include petroleum ether, hexane, chloroform, acetone, methanol, ethanol, benzene, ether, and carbon tetrachloride. All were satisfactory when absolute solvents were used with the exception of methanol, which destroyed viability of all seeds within 12 hr. Seed viability was also destroyed by all other solvents if 95% or weaker solutions of solvent were used. No adverse effects on seed germination were detected when the immersion period did not exceed 8 days and hand-threshed seed was used (*i.e.*, seed free of mechanical damage). When immersion was carried out for 60 days, viability dropped by 8 to 12%. Loss in seed viability was also much higher when machine-threshed seed was used (3 to 5% reduction for 8-day immersions and 12 to 20% reduction for 60-day immersions). Observations of single seed extractions in test tubes showed that seeds which did not germinate later could be assigned to two groups: seeds which released excessive amounts of oil to the solvent (6 to 20% of the original seed weight); and seeds which released only traces of oil. The former seeds, despite an intact outward appearance, were found to be internally cracked or damaged, permitting a deeper penetration of solvent with harmful effects on germination. Seeds in the latter case were immature, with poorly developed embryos or were small and lightweight. Because of the heavy contribution to an oil sample by the first group of seeds, it is conceivable that analytical data might be biased toward the mean of that group if its composition deviated significantly for some reason from the general sample mean.

The rate of oil removal did not increase in proportion to the length of immersion in solvent. After 6 days of immersion, the oil extracted from safflower and flax by chloroform was equal to 2.2 and 3.8%, respectively, of the original weight of these seeds. Assuming an oil content of 40% for both of these kinds of seeds, the amount of oil extracted represented 5.5% for safflower and 9.5% for flax of the total amount of oil in the seeds. Decanting the solvent daily and refilling the flasks with fresh solvent provided no significant increase in the amount of oil extracted.

Whether the technique described in this report is to be preferred over the one of cutting off and anlyzing portions of a seed depends on the objectives of each particular experiment. If a researcher needs to screen a few seeds, the proposed technique may not have an advantage. If, however, he needs to screen many samples, each consisting of several seeds, the proposed technique has obvious advantages, especially if the cut-off portions of seeds are to be sown months after the incisions were made. Prolonged storage of injured seeds leads to reduction of their viability, especially if proper seed storage facilities are lacking. Finally, the desirability of this technique with seeds other than the ones already mentioned may be influenced by whether components other than lipids are extracted by the solvent and interfere with the analytical procedures.

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Received for review January 13, 1972. Accepted June 7, 1972.