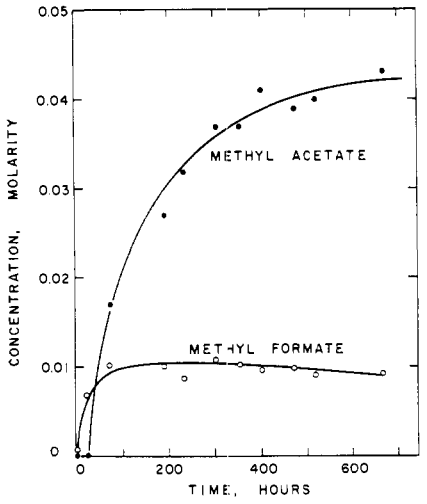

Figure 5. The formation of methyl acetate and methyl formate in whole smoke condensate as a function of time

initial period. Figure 5 also indicates that the rate of formation of methyl formate was extremely rapid and seemed to reach equilibrium after 70 hours. Methyl acetate, on the other hand, continued to form at a significant rate and had a concentration 4½ times that of methyl formate, even after a 700-hour period. Even though trace amounts of methyl acetate and methyl formate may be present in fresh smoke, the major portion results from secondary reactions which occur upon standing.

The formation of methyl acetate and methyl formate was followed in model experiments, using a mixture of methanol, formic acid, and acetic acid. The concentrations of the compounds used in the mixture were the same as those found in the smoke condensate. The quantities of methyl acetate and methyl formate formed and the rates of formation were comparable with those observed in the condensate.

The identification of methyl formate indicated the presence of formic acid in whole smoke condensate. The authors had been unable to detect this acid directly by the use of GLC. Positive identification of formic acid by GLC has not been accomplished because it decomposes on the hot metal inlet system of the apparatus (*9*). However, in other investigations on the composition of wood smoke, the presence of formic acid in substantial quantities has been established by the use of other methods (*4*, *5*, *7*, *8*).

Correlation studies between secondary reaction products and the organoleptic quality of food products treated with the condensate have not been made. The known odor qualities of the esters reported here, however, suggest the possibility that they may contribute to the development of a more mellow aroma of the smoke condensate upon standing.

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COMMUNICATIONS

ENZYME IDENTIFICATION

Nature of the Myrosinase Enzyme

Black, brown, and oriental mustard seeds were surveyed for myrosinase activity using methyl *n*-pentyl ketoxime-O-sulfonate (potassium salt) and sinigrin as the enzyme substrates. No component specific for the hydrolysis of oxime sulfonates was detected. Thus, the reported existence of a distinct enzyme, myrosulfatase, could not be confirmed.

THE ENZYME MYROSINASE is responsible for the development of the flavor and pungency of many food products, such as mustard and horseradish, by its hydrolysis of the thioglucosides. This enzyme has been considered to be a mixture of a thioglucosidase and myrosulfatase (*3*, *4*, *6*, *7*, *9*) in contrast to the concept of a single enzyme system (*2*, *5*, *8*) a thioglucosidase. The single enzyme theory describes the action of thioglucosidase on the mustard oil compounds as the hydrolysis of glucose from the thioglucoside followed by a Lossen rearrangement of the aglycone to give isothiocyanate and sulfate.

The work of Gaines and Goering (*3*, *4*) revived the controversy. These workers reported separating on diethylaminoethylcellulose (DEAE-C) the myrosulfatase and thioglucosidase com-

ponents of myrosinase from defatted Oriental yellow mustard seed, *Brassica juncea*. The myrosulfatase activity was determined by its action on sinigrin and other oxime sulfonates. Of the oxime sulfonates investigated, methyl *n*-pentyl ketoxime-*O*-sulfonate (potassium salt) was most susceptible to hydrolysis by myrosulfatase. Based on the results published by Gaines and Goering (*3, 4*), 14% of the total methyl *n*-pentyl ketoxime-*O*-sulfonate was hydrolyzed in 24 hours, while 35% of the sinigrin was hydrolyzed.

The authors' efforts to repeat the fractionation of a sample of Oriental mustard seed (*B. juncea*) were unsuccessful. The authors used DEAE-C and carboxymethylcellulose columns of the same dimensions as Gaines and Goering varying the source of the DEAE-C. Citrate buffer of 0.05*M*, 0.1*M*, and 0.2*M* was used. The elutions were done at room temperature, 10°, and 1° C. The authors also tried gradient elution using citrate buffer beginning at 0.05*M* and going to 0.3*M*. The pH of the 0.1*M* citrate buffer was changed from pH 6.2 to pH 6.0, pH 5.8, and pH 5.4. The samples applied to the columns were always prepared as by Gaines and Goering. In all the attempts of fractionation, the authors obtained a very active thioglucosidase fraction and never a myrosulfatase fraction.

Black (*B. nigra*), brown (*B. juncea*), and Oriental mustard seeds (*B. juncea*) were used to survey for the presence of myrosulfatase using methyl *n*-pentyl ketoxime-*O*-sulfonate as the enzyme substrate. Four different treatments were made to each species:

(*A*) Black, brown, and Oriental mustard seeds were ground in a Wiley mill. Five grams were placed in a mortar with 5 ml. of citrate buffer (0.1*M*, pH 6.2), ground slightly, washed into an Erlenmeyer flask with 5 ml. of citrate buffer, incubated on a slow shaker for 0.5 hour, and filtered through four layers of cheese cloth. The filtrate (0.2 ml.) was pipetted into a test tube containing 0.5 ml. of citrate buffer [pH 6.2, 0.1*M*, and 2.0 mg. of methyl *n*-pentyl ketoxime-*O*-sulfonate (potassium salt)] (*10*). The tubes were incubated for 24 hours at 36° C. The presence of sulfate was determined by precipitation as benzidine sulfate (*1*).

(*B*) Ground black, brown, and yellow mustard seeds were defatted with petroleum ether (30° to 60° C.) in a Soxhlet vessel for 12 hours. The defatted mustard seed was homogenized and dialyzed against distilled water for 24 hours. The preparation (0.2 ml.) was tested for activity as mentioned previously.

(*C*) The defatted mustard seed was precipitated with alcohol according to the directions of Sandberg and Holly (*9*). The dry protein powder (2.0 mg.) was tested for activity as outlined.

(*D*) Black, brown, and yellow mustard seeds were germinated for 48 hours in the dark and freeze-dried, and the dry germinated seeds defatted as previously outlined. The defatted product was homogenized and dialyzed for 24 hours against distilled water and tested as previously described.

In all these treatments, the activity of the preparations was tested by determining glucose and sulfate resulting from the hydrolysis of sinigrin. In all cases, at least 70% of the sinigrin was hydrolyzed.

When methyl *n*-pentyl ketoxime-*O*-sulfonate was used as the substrate, the sulfate was not detected at the end of the incubation. There appears to be no myrosulfatase activity in any of the four seed preparations. The presence of inactivators would be ruled out by dialysis and alcohol precipitation. The gentle treatment of the seed (case *A*) does not appear to allow the possibility of protein inactivation, when compared with the harsh treatment the protein received in the work of Gaines and Goering. Germination seems to activate many enzymes in the seed; however, germinated seeds showed no myrosulfatase activity.

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