Analysis of Glycosidically Bound 2,5-Dimethyl-4-hydroxy-3(2H)-furanone in Pineapple

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2,5-Dimethyl-4-hydroxy-3(2H)furanone (DMHF), also called "furaneol," was described as being "burnt pineapple"-like and is the major character impact compound of pineapple flavor concentrate (Rodin, et al., 1965). DMHF has been identified in pineapple (Rodin, et al., 1965), strawberry (Re, et al., 1973; Pickenhagen, et al., 1981), mango (Pickenhagen, et al., 1981) and arctic bramble (Kallio, 1976). The stability of DMHF has been studied (Hirvi, et al., 1980; Shu, et al., 1985). It is somewhat unstable in air and in an aqueous solution. Hirvi, et al. (1980) reported that furaneol can be totally destroyed during the processing or canning of fruits such as pineapple. On the contrary, Lee and Nagy (1987) noted that canned pineapple samples showed the presence of relatively high amounts of DMHF as compared with fresh pineapple juice, and that for grapefruit juice, increasing the temperature and storage period caused more DMHF to form and accumulate. They attributed their observations to the formation of DMHF from the degradation of sugars either during processing or during subsequent storage.

This paper reports evidence of the presence of glycosidically bound DMHF in pineapple. In the last few years the analysis of flavor precursors and intermediates, especially glycosides in fruits, has received increasing interest and attention (Strauss, et al., 1986; Schwab and Schreier, 1988).

Materials and Methods

Material: The 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) was kindly provided by Firmenich Inc. (Princeton, NJ). Amberlite XAD-2 (20-60 mesh) and γ-valerolactone were purchased from Aldrich Chemical Co. (Milwaukee, WI). Almond β-glucosidase was obtained from Sigma Chemical Co. (St. Louis, MO). All solvents were HPLC grade from Fisher Scientific Co. (Springfield, NJ).

Fresh pineapple (*Ananas Comosus* L. Merr.) grown in Hawaii and Costa Rica were purchased from a local market. The fresh clear juice was prepared from skinned, cut and cored whole fruit. It was crushed and filtered through celite 545 (J. T. Baker Chemical Co., Phillipsburg, NJ).

Samples prepared for HPLC: Enzymic hydrolysis of glycosidically bound aroma compounds in juice was accomplished by adding 20 mg (5.3 unit/mg) of almond β-glucosidase to 40 ml fresh clear pineapple juice and incubating for 72 hrs at 37°C.

40 ml of pineapple juice, with or without enzymic hydrolysis was extracted three times with 40 ml of ethyl acetate. The combined extract was dried over anhydrous sodium sulfate and concentrated by nitrogen gas to 1 ml.

High performance liquid chromatography: Quantitative HPLC determinations were conducted with a Varian 5000 liquid chromatograph, 2050 variable wavelength detector and CDS 401 computing inte-

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grator. A reversed phase PartiSphere C_{18} column (Whatman Inc., Clifton, NJ) was used and the mobile phase was 0.05 M sodium acetate (pH 4.0)/ methanol (80:20). Other operating conditions were flow rate, 0.5 ml/min; UV, 290 nm; chart speed, 0.2 cm/min and injection volume, 10 μl.

Separation of free and bound aroma compounds: A sample of 805 ml of Costa Rican pineapple clear juice was passed through an Amberlite XAD-2 column (1 cm x 50 cm) with a flow rate of 2.0 ml/min. The column was rinsed with 100 ml of water to eliminate sugars, acid and other water-soluble compounds. The free fraction of the aroma fixed on the column was eluted using 800 ml of pentane-ether (1:1) at a flow rate of 2.0 ml/min. The pentane-ether extract was dried with anhydrous sodium sulfate and then concentrated to a final volume of 0.2 ml with nitrogen. The bound fraction was subsequently eluted using 800 ml of methanol. It was then concentrated to dryness using a stream of nitrogen and dissolved in a 120 ml solution of 0.2 M citric-phosphate buffer (pH = 5). The mixture was washed twice with 100 ml of pentane-ether to eliminate possible traces of the free fraction. Almond β-glucosidase (120 mg; 5.3 unit/mg) was then added to the extract which was incubated at 37°C for 72 hrs. The medium was then extracted three times with 120 ml of dichloromethane, dried over anhydrous sodium sulfate and concentrated to a final volume of 0.2 ml with a stream of nitrogen. γ-Valerolactone was added as internal standard to both free and bound fractions before concentration.

Gas chromatography: A Varian 3400 gas chromatograph equipped with a fused silica capillary column (50 m x 0.32 mm i.d., 1.05 μm thickness, HP-1; J & W) and a flame ionization detector was used to analyze the furaneol in free and bound fractions. The operating condition were as follows: injection temperature, 270°C; detector temperature, 300°C; injection volume, 0.4 μl; helium carrier flow rate, 1.0 ml/min; temperature program, 40-260°C at 2°C/min and held at 260°C for 20 min. A split ratio of 50:1 was used.

Results and Discussion

Glycosidically bound components of Costa Rican pineapple juice were isolated by Amberlite XAD-2 column according to the method of Gunata, et al. (1985). After enzymatic hydrolysis of the bound fraction by almond β-glucosidase, the aglycons released were extracted with dichloromethane. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone was positively identified to be presented as major component in the extract by gas chromatography and gas chromatography-mass spectrometry.

For the quantification of DMHF in pineapple juice, the high performance liquid chromatographic

method developed by Lee and Nagy (1987) was used. Both Hawaiian and Costa Rican pineapples available from the local supermarket were used for the study. The DMHF contents of the fresh Hawaiian pineapple juice and Costa Rican pineapple juice were determined to be 2.34 and 1.59 mg/L. After incubation of the pineapple juice samples with almond β-glucosidase, the DMHF contents of the sample raised to 6.04 and 4.59 mg/L for Hawaiian and Costa Rican pineapple respectively.

From this study, it is clear that the 2,5-dimethyl-4-hydroxy-3(2H)-furanone glycoside is the precursor of DMHF in pineapple. During the canning process, the free DMHF may be decomposed; however, the glycosidically bound DMHF will be thermally hydrolyzed to yield free DMHF. This may be the reason that Lee and Nagy (1987) did not observe the loss of DMHF during processing.

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