a 17.4 mm flat seat metal rupture disc type B with a pressure rating of 100 psi at 150 C (BS and B Safety Systems, Tulsa, Okla.) be inserted into the reactor cover in case excess pressure is introduced or generated.

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An Apparatus for the Measurement of Lipoxygenase Activity and Pentane Production[1]

ABSTRACT

The enzyme lipoxygenase has been demonstrated to produce pentane. However, until now no apparatus has been available to measure the pentane concomitantly with the lipoxygenase activity. Such an apparatus is described by this paper. The apparatus consists of a reaction vessel which can be sealed with a rubber septum to allow sampling for pentane production and an oxygen measuring device to determine the oxygen level of the system. The apparatus is being used to conduct in-depth studies of the lipoxygenase enzyme and its possible role in food quality.

INTRODUCTION

Pattee, et al., (1) demonstrated that five major volatile compounds were produced during maturation of the peanut seed, with pentane production dominant during the middle and late maturation stages. They postulated that pentane is produced in a reaction sequence starting with the action of lipoxygenase on linoleic acid. To study this further a simple apparatus for the measurement of both lipoxygenase activity and pentane production was developed.

EXPERIMENTAL PROCEDURES

Lipoxygenase activity was measured by O_2 consumption by the polarographic method of Mitsuda, et al., (2) and pentane production was determined concomitantly at room temperature (25 C ± 2 C) in a glass vessel fitted with a Clark oxygen electrode and a serum stopper (Fig. 1). The vessel

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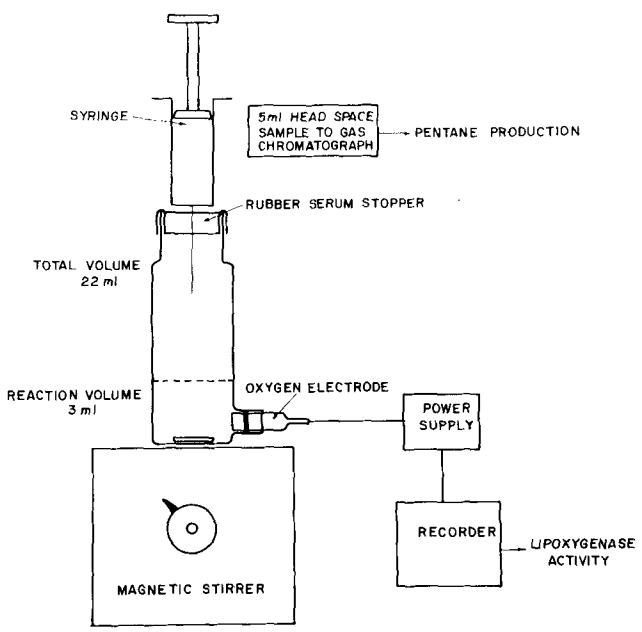

FIG. 1. Apparatus for the measurement of lipoxygenase activity and pentane production.

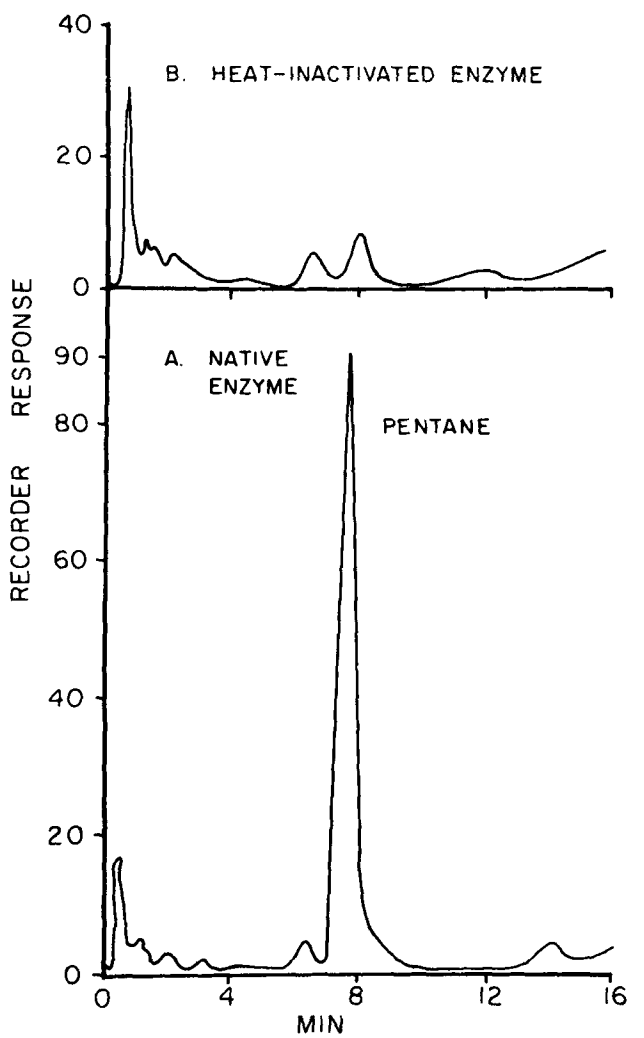

FIG. 2. Gas chromatograms of the volatile reaction products produced from the oxidation of linoleic acid by soybean lipoxygenase; (A) 25 ng crude commercial enzyme and (B) 25 ng heat-inactiviated enzyme.

contained 11.9 μmoles linoleic acid and 2.8 ml 0.1% Tween 20 in 0.1 M borate buffer, pH 9.0. The reaction was started by the addition of 0.2 ml commercial (Nutritional Biochemicals, Cleveland, Ohio) soybean lipoxygenase (0.25 mg/ml in 0.01 M phosphate buffer pH 7.0) and a serum stopper pierced by a 16 gauge needle was fitted quickly on the reaction vessel. This ensured that no pressure changes occurred in the vessel, and the needle was removed immediately after the serum stopper was secured. Under these conditions it required 1.5-2 min for the available oxygen in the reaction medium to be depleted by the lipoxygenase, and oxygen consumption was linear with time and enzyme concentration. At a predetermined time after achieving anaerobic conditions, a 5 ml gas volume was withdrawn with an air-tight syringe and injected into a Varian Aerograph Gas Chromatograph model 1840. A Chromosorb 102 column (6 ft x 1/8 in. stainless steel), operated isothermally at 140 C, was used to separate the volatile compounds. Under the above assay conditions the major peak previously had been identified as pentane by combined gas chromatographic mass spectral analysis (1,3). Peak areas were integrated using an Infotronics CRS-100 digital readout system, and pentane data are presented as integrator area units. Chromatograms of typical and control (heat inactivated enzyme) reactions show that pentane was not produced without native enzyme (Fig. 2). Pentane production using this apparatus was linear 5-20 min (Fig. 3), the delay in linear production being the time necessary for depletion of oxygen from the system. These results confirm the observation of Garssen, et al. (5) in regard to the necessity of anaerobic conditions for the production of pentane at pH 9. Previously, we reported (4) that pentane production is linear with enzyme concentration and has an optimum substrate concentration of 2.1 mM and an optimum pH of 9.

Garssen, et al., (5) and Johns, et al., (4) have found pentane, and St. Angelo, et al., (6) has found hexanal to be the principal secondary reaction products formed by the enzymatic oxidation of linoleic acid by soybean and peanut lipoxygenase, respectively. Peanut (6) and soybean (7) lipoxygenases have been reported to be specific for the C-13 position of linoleic acid, which indicates that the specificity of the secondary reaction is different for the two enzymes. The technique described in this paper could, thus, prove useful in determining whether only hexanal is produced by the peanut enzyme.

This method is rapid, requiring only 20-25 min for each lipoxygenase and pentane assay, and can be easily used or modified for use in measuring any reaction or sequence of reactions where oxygen is consumed or liberated and a volatile product is formed.

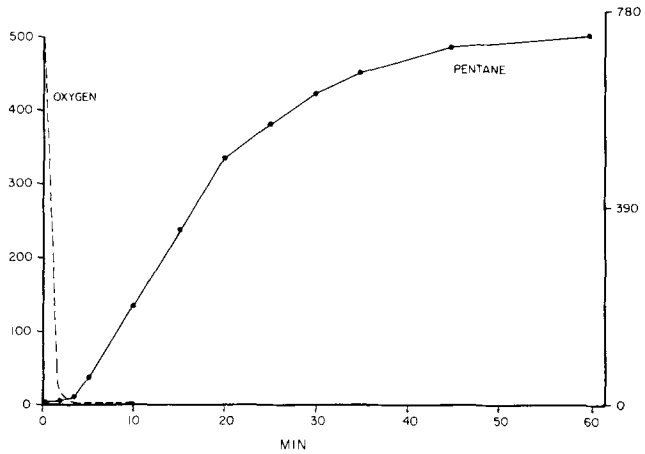

FIG. 3. Pentane production and oxygen consumption as a function of time. (————) pentane: area units x 10^{-3}, (-----) nmoles oxygen.

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Infrared Absorption of Methyl *cis*-9,*trans*-11-, and *trans*-10,*cis*-12-Octadecadienoates

ABSTRACT

The ratio of absorptivity at 10.2 μm and 10.6 μm differs between methyl *cis*-9,*trans*-11-, and *trans*-10,*cis*-12-octadecadienoates. For the *cis*-9,*trans*-11-ester, $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ is in the range of 1.1-1.2; for the *trans*-10,*cis*-12-ester, it is 1.3-1.4. These differences in absorptivities are great enough to affect significantly compositions calculated from IR absorption.

INTRODUCTION

The two bands at 10.2 μm and 10.6 μm in the IR absorption spectrum of conjugated *cis,trans*-linoleate isomers have been well established since they were reported by Jackson, et al., (1) in 1952 and have been used for quantitative determination of conjugated *cis,trans*-and *trans,trans*-isomers (2). This quantitative procedure, which has been described in a number of reviews (3-7), assumes that the absorptivities at the maximum of the two bands are the same for all the *cis,trans*-isomers in the sample. Qualitatively the ratio of absorptivities of the two bands $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ also has been used as an indication of purity. Chipault and Hawkins (2), finding a higher ratio for the *trans*-10,*cis*-12 isomer than the *cis*-9,*trans*-11, stated that, although the two compounds may have different