LIBERATION OF FATTY ACIDS DURING FERMENTATION OF PEANUT PRESS CAKE

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ABSTRACT

Free fatty acids liberated during fermentation of peanut press cake were isolated, separated, identified, and quantitatively analyzed. Fermentations were conducted with pure cultures of *Neurospora sitophila* and *Rhizopus oligosporus* at 30°C for 72 hours. Separation and identification of esterified free fatty acids were accomplished using a gas chromatograph connected to a mass spectrometer through a jet separator.

Mass spectra of methyl esters of fatty acids showed a base peak at m/e 74, and all esters were identified as methyl-myristate, methyl-palmitate, methyl-stearate, methyloleate, methyl-linoleate, methyl-linolenate, methyl-arachidate, methyl-behenate, and methyl-lignocerate, respectively.

Myristic, linolenic, arachidic, behenic, and lignoceric acids were not detected in uninoculated cake; while, palmitic, stearic, oleic, and linoleic acids totally were present at a level less than 0.6%. As the fermentation progressed, more fatty acids were liberated from oil tryglicerides. The order of liberation of major fatty acids appeared to be oleic, linoleic, palmitic, and stearic acids.

The rate of fatty acids liberation in cake fermented by *R. oligosporus* was much higher than that in cake fermented by *N. sitophila.* Total free fatty acid content of oil in the cake fermented by *R. oligosporus* increased from 0.6 to 39%; while, in the cake fermented by *N. sitophila,* the increase was only from 0.6 to 10% after 72 hours of fermentation.

INTRODUCTION

Traditional food fermentations are characterized by their simplicity and rapidity. Oncom which is a fermented product prepared from peanut press cake is an example. The product is very popular in Western Java where it has been prepared and consumed for centuries. In oncom preparation, the peanut press cake is first broken up either by hand or with a knife, and then soaked overnight, washed, pressed, steamed, cooled to room temperature, inoculated with *Neurospora sitophila,* wrapped with banana leaves, and inoculated at room temperature for 2 or 3 days.

As the fermentation progress, the biochemical changes take place in oncom due to the activity of enzymes produced by the mold. Perhaps, the alteration of lipid components of peanuts is the most notable changes. The investigation was conducted to monitor the type and quantity of fatty acids liberated from peanut lipids during the fermentation.

MATERIALS AND METHODS

Materials

Peanut was purchased from local stores. In the preparation for fermentation, the peanut was broken down into small pieces and then pressed to get peanut press cake. Mold cultures used in the experiment were *N. sitophila* ATCC 14151, *R. oligosporus* ATCC 22959, and *N. sitophila* isolated from local oncom variety.

Methods

Preparation of oncom fermentation

Oncom was prepared using the method as described by Fardiaz and Markakis (1981). Fig. 1 shows the processing steps done in the laboratory.

Free fatty acid extraction

The method of Mattick and Lee (1959) was used to extract free fatty acids from the sample. Oil was first extracted from the sample with diethyl ether using a Gold-fisch extractor. One g of oil and 8 mg of n-heptadecanoic acid (internal standard) were transferred into a 60 ml separatory funnel. Thirty five ml of a mixture of diethyl ether and petroleum ether (1 : 1) were added to dissolve the oil, then, 6.5 ml of 95% ethanol and 12.5 ml of 1% Na_2CO_3 were added. The mixture was shaked several times and the aqueous layer containing the sodium salts of free fatty acids was separated into another 60 ml separatory funnel.

Extraction of free fatty acids from the ether layer was repeated three times; first, with 1.5 ml of 95% ethanol and 7.5 ml of 1% Na_2CO_3; second, with 1.5 ml of 95% ethanol and 5.0 ml of 1% Na_2CO_3, and finally with 6.5 ml of distilled water. All the aqueous phases were collected and combined, whereas, the ether layer containing the glycerides was discarded.

To a separatory funnel containing the aqueous layer 1.5 ml of 10% H_2SO_4 was added in order to free the fatty acids. The free fatty acids were then extracted with 12.5 ml of the solvent mixture mentioned above. The ether layer was separated and transferred through Whatman no. 1 filter paper containing several g of anhydrous Na_2SO_4 into a 5 ml screw-cap vial. The solvent was evaporated to dryness by passing a stream of nitrogen gas through the vial. The extraction was repeated three times with fresh solvent.

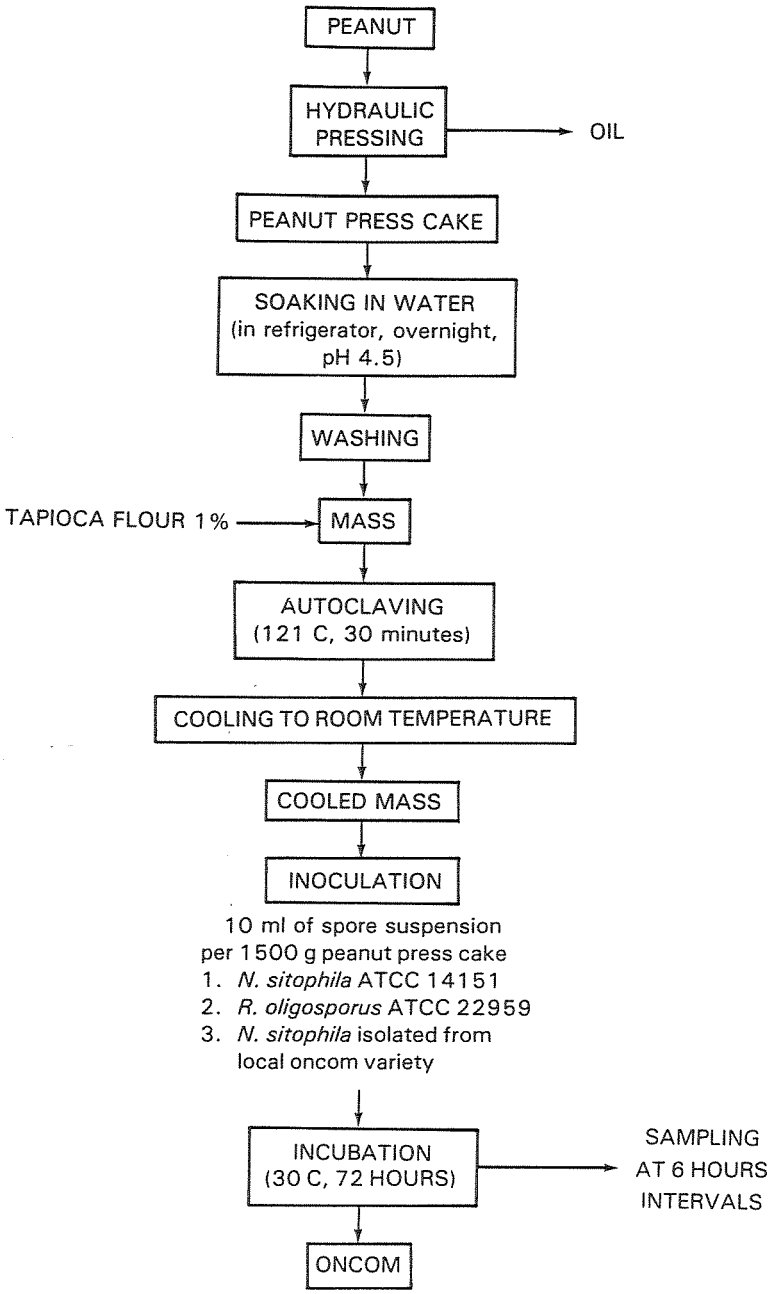


Fig. 1. Processing diagram of oncom preparation.

Esterification

Free fatty acids were converted to their methyl-esters prior to GLC analysis because these derivatives are more volatile than the acids. Boron trifluoride in methanol (14%, w/v) was used as esterifying reagent according to the method of Supelco, Onc. (1975).

Into the vial containing dry free fatty acids, 2 ml of benzene was added to dissolve the acids. Two ml of BF_3-methanol was further added into the vial and mixed well. The vial was placed in a small beaker with water and boiled for 3 minutes on a steam bath. To stop the reaction, 1 ml of distilled water was added to the reaction mixture, which was then separated into two layers. The top layer contained the methyl esters dissolved in benzene, while the bottom layer was a mixture of methanol, water, and acid catalyst. To separate the two layers, the vial was centrifuges, and the benzene layer was transferred with a syringe into another vial. Two microliter of the benzene containing methyl esters was injected into the gas chromatograph using a 10 microliter Hamilton syringe no. 701 (Hamilton Co., Reno. NEV.).

Gas chromatograph/mass spectrometer condition

The GC/MS used was a Hewlett Packard 5840A Gas chromatograph/HP 5985 Mass Spectrometer (Hewlett Packard Corp., Avondale, PA.). The column was a 6 ft. × 0.250 in. o.d. glass column packed with 3% DEGS on 80/100 mesh chromosorb. The helium flow rate was 25 ml per minute, and the temperature was programmed from 130 to 190°C at 10°C per minute. The ion source and analyzer temperatures of the mass spectrometer was maintained at 200°C. The accelerating voltage was 2000 V, ionizing potential 70 eV, repetitive scan 266.7 a.m.u. per second, and scan time 1.4 seconds.

RESULTS AND DISCUSSION

During the first 12 hours, the growth of both *N. sitophila* and *R. oligosporus* progressed slowly. After 18 hours, a rapid growth of *R. oligosporus* became obvious where the mycelial growth penetrated deeply into the peanut press cake mass, forming a compact semisolid product. Unlike *R. oligosporus,* the growth of *N. sitophila* was slow. It took 24 hours for this mold to develop obvious mycelial growth.

Gas Chromatograph/Mass Spectrometer Analysis

Fatty acids extracted from the sample were converted to their methyl esters prior to gas chromatograph analysis because of the difficulties encountered in their separation by gas chromatograph. Identification of methyl esters of fatty acids was done in a mass spectrometer connected to the gas

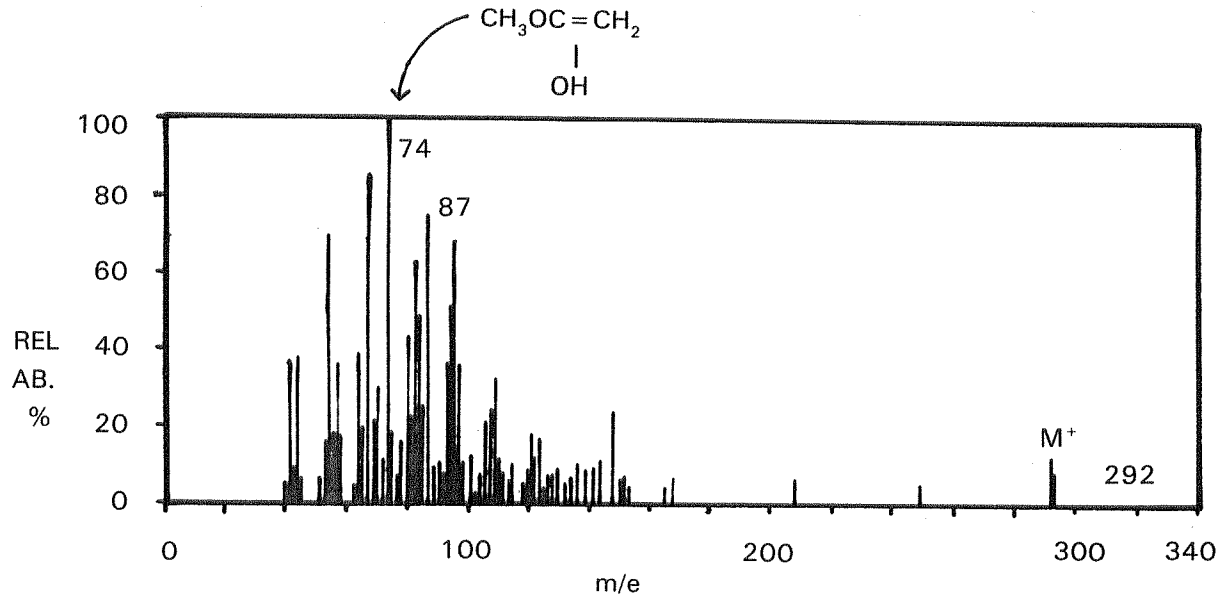
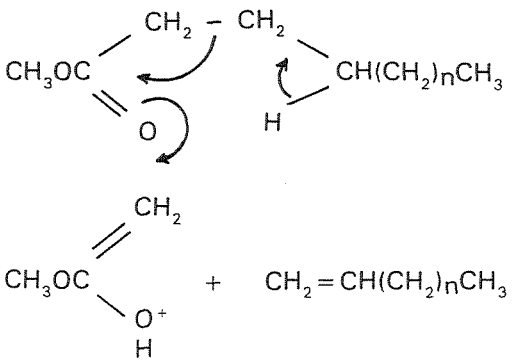


Fig. 2. Mass spectra of methyl linolenate (F.W. 292.5).

chromatograph through a jet separator. The efficiency of free fatty acid extraction was evaluated by spiking internal standard (n-heptadecanoic acid) to peanut oil prior to extraction. The recovery of added internal standard in each extraction ranged from 91 to 96%.

Fig. 2 shows mass spectra of methyl linolenate. The molecular ion peak for methyl linolenate is mass 292 as seen in the figure. The isotope peak at mass 293 (M + 1) is due primarily to the 1.08% carbon 13 in natural carbon.

The base peak, the most intense peak in the spectrum, of methyl linolenate occurred at mass 74. As described elsewhere, this peak was formed by the McLafferty rearrangement which involves the formation of a six-membered ring with transfer of a hydrogen from the number four carbon atom to the carbonyl oxygen followed by cleavage of the 2—3 carbon to carbon bond, as follows. In addition, a series of peaks having masses of 87, 101, 115, 129, 143, 157, were found as results of simple fragmentation which produced ions with the general formula $CH_3O_2C(CH_2)_n$. All the esters identified had the same base peak at m/e 74 and a large peak at m/e 87.



Fatty Acids Liberated During Fermentation

Four peaks which corresponded to methyl esters of palmitate, stearate, oleate, and linoleate were detected in the control-uninoculated cake. Prior to fermentation the uninoculated cake contained 5.33 mg of total free fatty acids per g. However, during 72 hours fermentation, the total free fatty acids content of the cake increased approximately three times. Linoleic acid was the dominant free fatty acid which contributed up to 50% of total free fatty acids found in the uninoculated cake.

As the fermentation progressed, more fatty acids were liberated from the peanut oil triglycerides. Figs. 3 and 4 show the gas chromatograph elution pattern of free fatty acids liberated during 72 hours fermentation of peanut

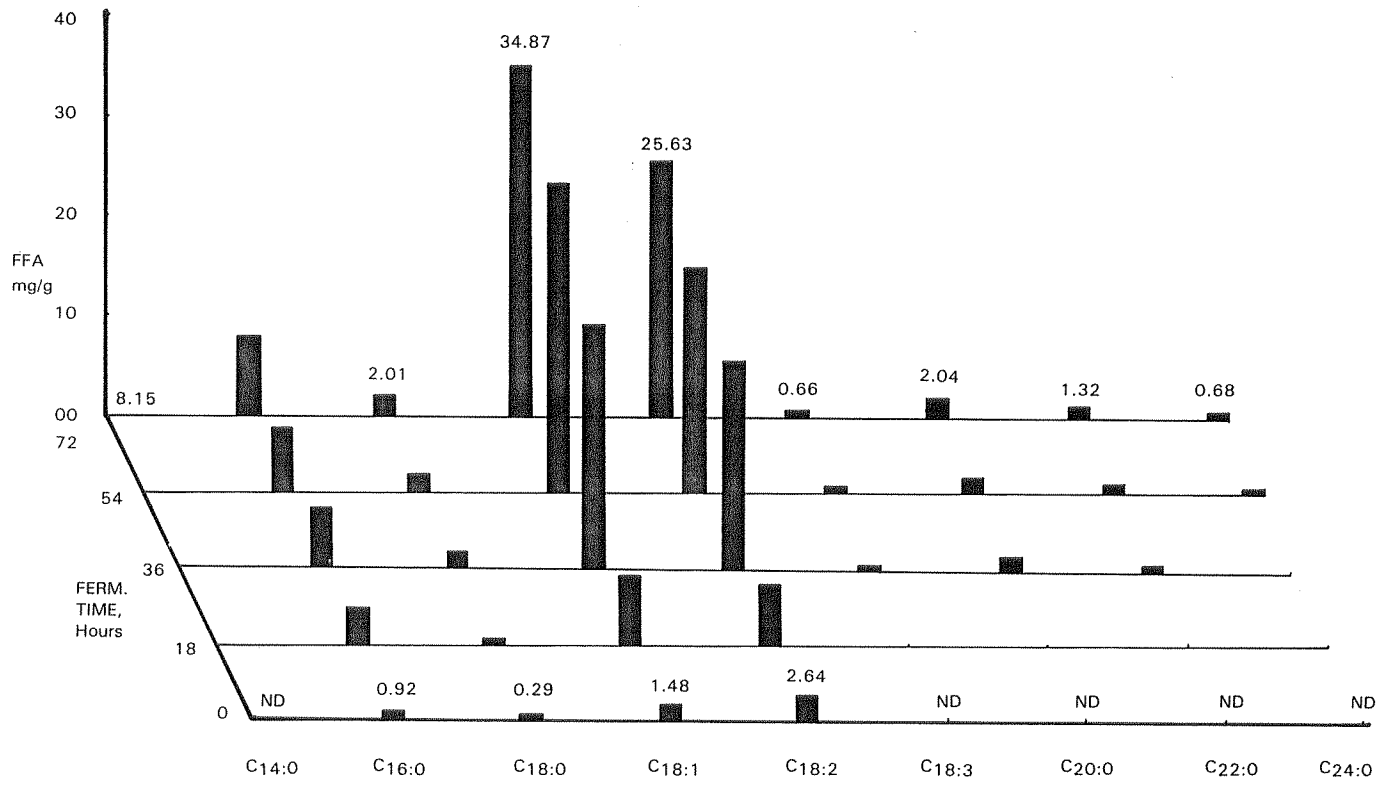


Fig. 3. Pattern of free fatty acids liberated during fermentation of peanut press cake by *Neurospora sitophila.*

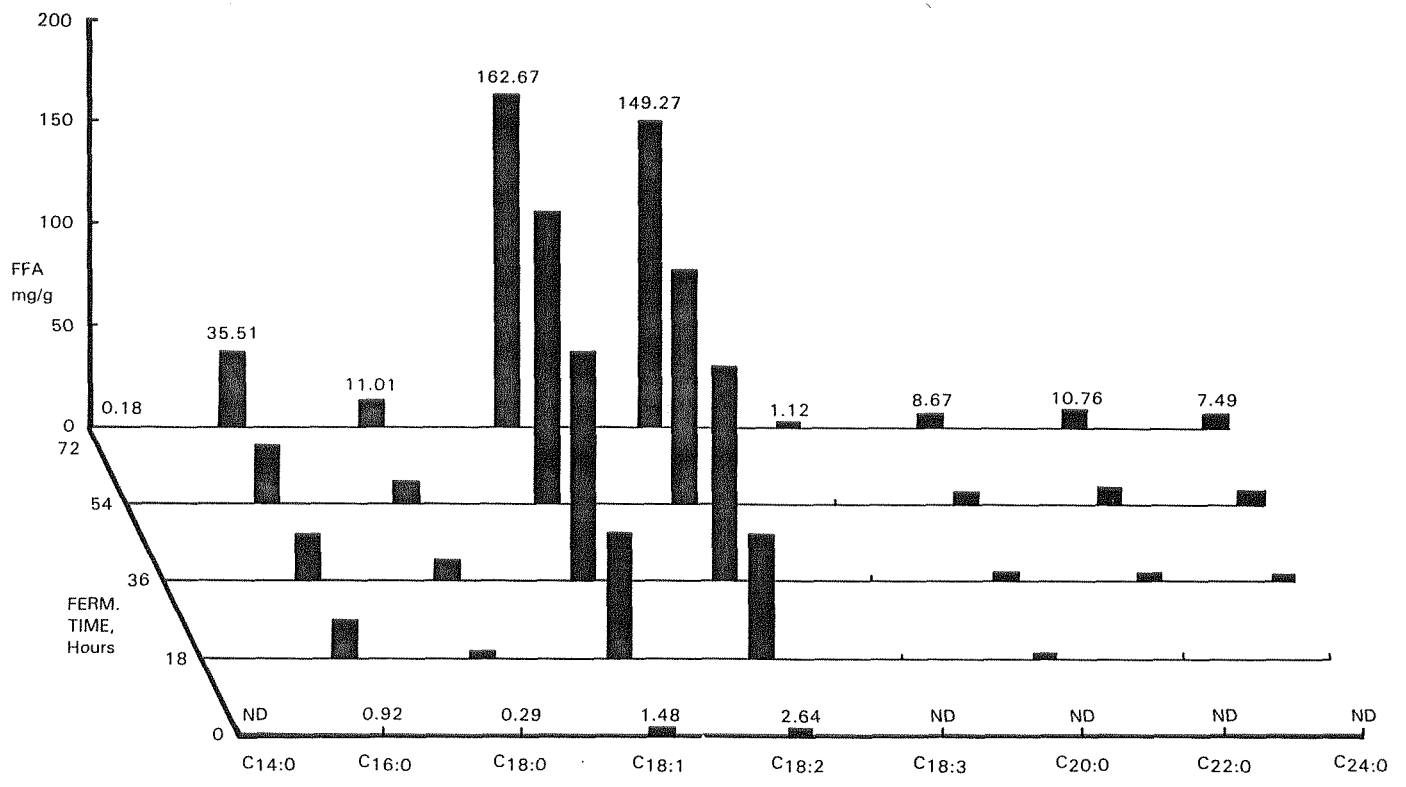


Fig. 4. Pattern of free fatty acids liberated during fermentation of peanut press cake by *Rhizopus oligosporus*.

press cake by *N. sitophila* and *R. oligosporus*, respectively. Both patterns are the same except for the magnitude of fatty acids liberated from the peanut oil triglycerides. The order of liberation of major fatty acids appeared to be oleic, linoleic, palmitic, and stearic acids. This order is apparently similar to that occurred in groundnut oil infected by *Aspergillus* (Tomlins and Twonsend, 1968).

Figs. 3 and 4 also indicate that *R. oligosporus* liberated fatty acids at a much higher rate than *N. sitophila* did. After 72 hours of fermentation, nine free fatty acids consisted of myristic (C_{14}:0), palmitic (C_{16}:0), stearic (C_{18}:0), oleic (C_{18}:1), linoleic (C_{18}:2), linolenic (C_{18}:3), arachidic (C_{20}:0), behenic (C_{22}:0), and lignoceric (C_{24}:0) acids were detected in all samples. It was found that in peanut press cake fermented by *N. sitophila,* the total free fatty acids

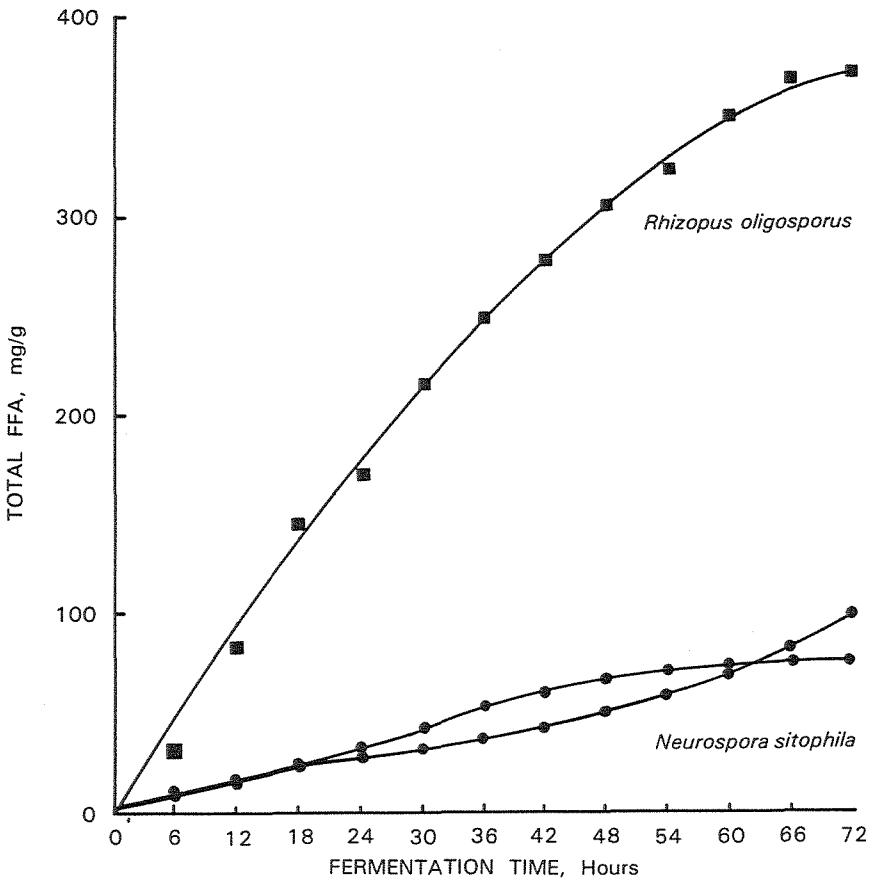


Fig. 5. Total free fatty acid liberated during fermentation.

content of oil increased from 0.6 to 10%; while, in peanut press cake fermented by *R. oligosporus* the increase was from 0.6 to 39% after 72 hours of fermentation (Fig. 5). This finding agreed with results reported by Beuchat and Worthington (1974) who monitored lipolytic activity of different molds by standard alkali titration of extracted oil.

In this experiment, substrates and environment conditions were uniform in all treatments; therefore, the difference in fatty acid liberation was mainly due to different specific ability in producing lipase between two molds. Indeed, *R. oligosporus* was highly lipolytic as reported by Alford *et al.* (1964) who found that from 82 microorganisms studied only 13 were highly lipolytic included *R. oligosporus.*

Based on common position of fatty acids in peanut oil triglycerides, order of fatty acid liberation during the fermentation of peanut press cake indicates that the lipase produced by the mold was typical pancreatic-type 1—3 lipases which liberated fatty acids much more rapidly from the 1- and 3- positions of a triglyceride than from the 2-position.

It is possible that liberation of fatty acids increases the digestibility of peanut lipids. Besides, free fatty acids may contribute to the development of typical oncom flavor.

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