Technical

Distribution of Oil or Other Diethyl Ether Extractable Material in Various Fractions of Peanuts Infected with Selected Fungi

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ABSTRACT

Percentage oil or other diethyl ether soluble material was determined in lyophilized ground whole seeds and in buffer (sodium phosphate; pH 7.9, I - 0.01) soluble and insoluble extracts of high quality "Florunner" peanuts, (*Arachia hypogaea* L.) not inoculated or inoculated with *Aspergillus parasiticus, Aspergillus oryzae, Rhizopus oligosporus,* or *Neurospora sitophila* and held for various time intervals to 18 days. During the test period, percentage ether extractable material increased in buffer soluble fractions of peanuts infected with these fungi and decreased in insoluble preparations. The trends of these quantitative changes at various test intervals were similar for seeds infected with *A. parasiticus* and *R. oligosporus.* Likewise, percentages were similar for seeds infected with *N. sitophila* and *A. oryzae.* The percentage ether soluble material in whole seeds infected with these fungi either decreased slightly or did not change during the test periods. Data suggest that techniques developed to separate oil and protein fractions from high quality peanuts using aqueous extraction processes may not be suitable for isolating these components from molded or partially decomposed seeds.

INTRODUCTION

Research to increase the utilization of peanuts as a source of protein for food and beverage fortification has led to the development of techniques which may be industrially applicable for the production of meals, flours, concentrates, and isolates (1-5). Aqueous milling processes which simultaneously recover oil and protein from peanuts are especially promising because they do not involve the use of organic solvents or excessive heat, both of which may irreversibly alter functional and nutritional properties of proteins (2,5).

Several researchers have conducted studies on methods for removal or detoxification of mycotoxins in oilseeds and meals (6-10). Studies are currently in progress to develop and evaluate procedures for the extraction and separation of proteins and oils from peanuts which are otherwise considered inedible due to the presence of aflatoxigenic or non-aflatoxigenic aspergilli (11-14). Such procedures would ideally yield high quality, food grade proteins, free from constituents which may be potentially harmful to human health. Concern has therefore emerged as to whether newly proposed as well as standard techniques which have been developed in various laboratories for fractionating oil and protein from high quality peanuts using aqueous milling processes can be applied to separate the same components from peanuts and meals infected with various fungi.

Studies on molecular changes occurring in fermenting soybeans and peanuts have shown that fungi actively hydrolyze lipids to free fatty acids (FFA) (15-20). Also, studies to determine the influence of various environmental conditions on the development of *Aspergillus flavus* on inoculated peanuts showed that hydrolysis of oil to FFA closely paralleled growth, sporulation, and aflatoxin production by the fungus (21-23). Moreover, proteins soluble in aqueous extracts of peanuts inoculated with *A. parasiticus* or *A. oryzae* were degraded to small polypeptides, free amino acids, and/or insoluble components (11-14). Therefore, it would be desirable to gain information pertaining to changes in peanut oil and protein at the molecular level prior to assessing the need for process modifications for their extraction on an industrial scale. This paper reports data on changes in the relative percentage distribution of oil or other ether extractable material in buffer soluble and insoluble extracts of peanut seeds infected with various fungi.

MATERIALS AND METHODS

Aspergillus parasiticus NRRL A-16,462, *Aspergillus oryzae* NRRL 1988, *Neurospora sitophila* NRRL 2884, and *Rhizopus oligosporus* NRRL 2710 were grown on potato dextrose agar slants at 24 C for 10 days. Conidia (or spores) were removed from each culture surface by gently washing with 0.005% Span 20 in sterile water. Testa-free "Florunner" peanuts were then submerged in the conidia suspension for 1 min, drained, and placed in petri dishes. Inoculated and uninoculated peanuts were incubated in a high humidity chamber at 29 C for various times ranging to 18 days. Two independent trials were performed for each test fungus (experiments 1 and 2). In experiments with *A. parasiticus* and *A. oryzae,* duplicate sets of three inoculated and three control seeds were collected at days 2, 4, 7, 9, 11, and 18. Mycelia and conidia, if present, were removed from the seed surface at each time interval and saved for further analysis. Peanuts infected with *N. sitophila* and *R. oligosporus* were similarly collected at days 2, 4, and 7. Uninoculated and inoculated whole seeds and mycelia/conidia or mycelia/spore mixtures were ground in pH 7.9 (I = 0.01) sodium phosphate buffer using a mortar and pestle and centrifuged at 43,000 x g for 30 min. Centrifugation separated ground extracts into three portions consisting of a fat pad (top layer), a buffer soluble extract (aqueous middle layer), and a pellet of insoluble material. The soluble extract was carefully poured from the centrifuge tube, and the remaining fat pad and pellet were combined. These soluble and insoluble fractions and unfractionated control and inoculated seeds were lyophilized. Portions or dried

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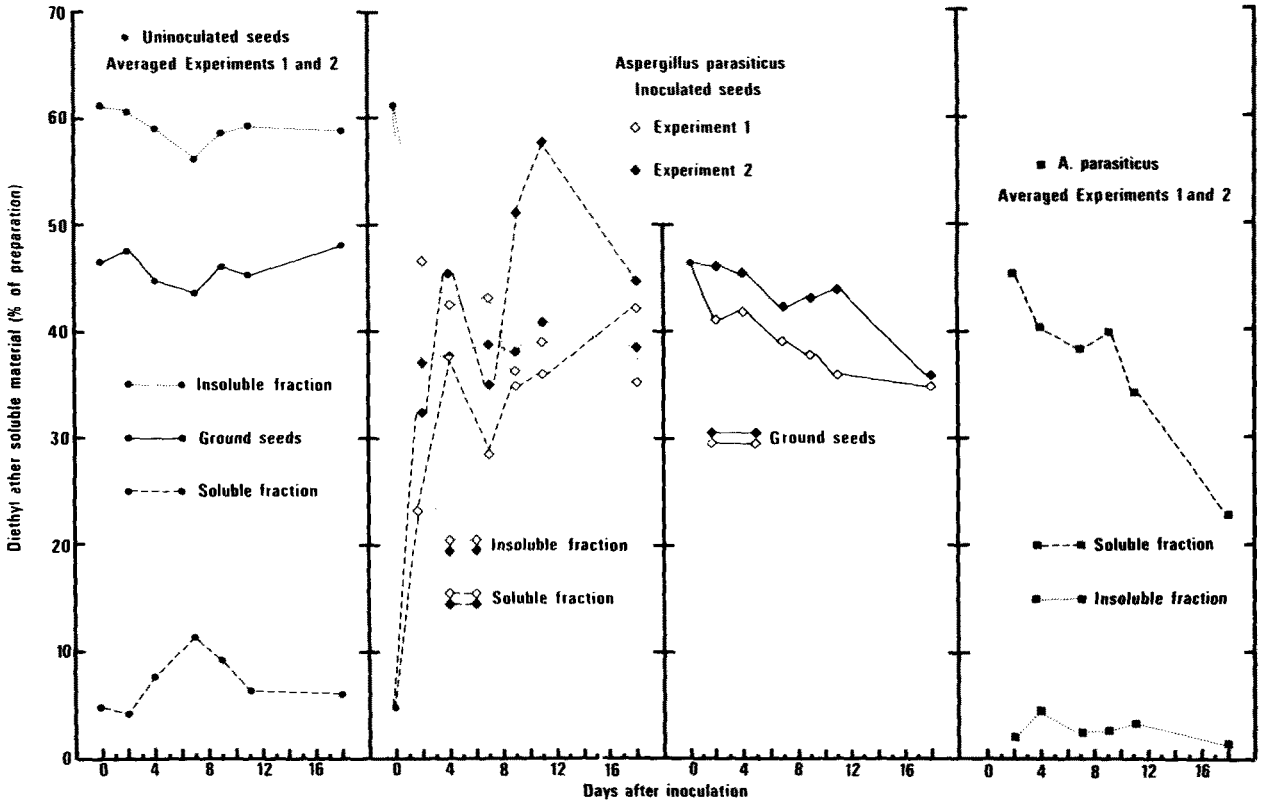

FIG. 1. Percentage diethyl ether soluble material in lyophilized ground seeds and buffer soluble and insoluble extracts of peanut seeds and fungal growth (mycelia/conidia). Seeds were not inoculated or inoculated with *Aspergillus parasiticus* for various time intervals to day 18. Fungal material used in these analyses was collected from the surface of infected seeds.

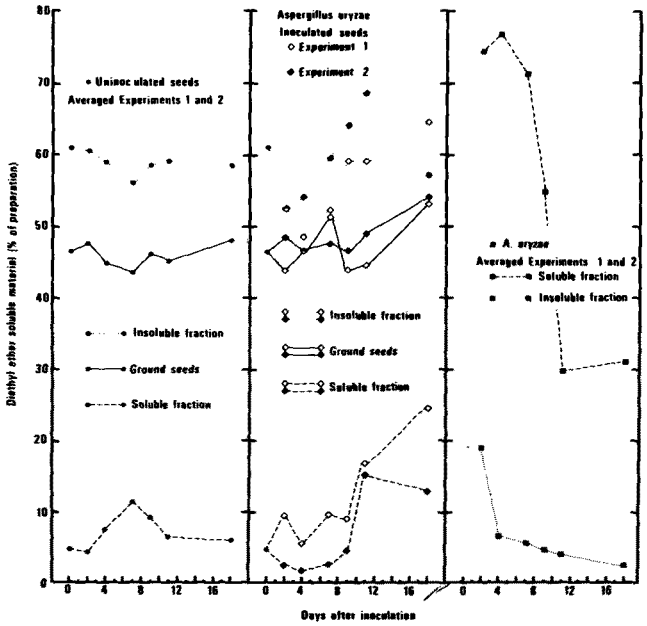

FIG. 2. Percentage diethyl ether soluble material in lyophilized ground seeds and buffer soluble and insoluble extracts of seeds and fungal growth (mycelia/conidia). Seeds were not inoculated or inoculated with *Aspergillus oryzae* for various time intervals to day 18. Fungal material used in these analyses was collected from the surface of infected seeds.

samples ranging from 0.1 to 1.0 g were ground in 20 ml of ether and allowed to stand for 18-20 hr in sealed vials. Ten-milliliter aliquots of clarified ether extracts were evaporated under a vented hood in tared vials, dried at 65 C for 18-20 hr, weighed, and the percentage ether soluble material in the test fractions was determined. Unpublished data from our laboratory indicate that this method of quantitation of ether soluble material in peanuts compares favorably to the Soxhlet extraction procedure (C.T. Young, private communication, 1975).

RESULTS

Data from all experiments involving the four test fungi were subjected to analysis of variance. Since in many cases the experiment, treatment, time, and fraction variables, in addition to their first and second order interactions, were significant ($P<0.05$), averaged data from two experiments for each of the test organisms are presented in Figures 1-3. The percengage of ether soluble material from control seeds and fungal mycelia or spores shown in these figures were averages of both experiments. The material from inoculated seeds soluble in ether was not specifically characterized and thus could contain oil and other lipids. Only small quantities of nonlipid materials from fermented peanut meal have been shown to partition into ether (20). However, until these components are properly identified, the fractions from infected seeds will be referred to as ether soluble materials.

The percentage lipid in buffer insoluble fractions of uninoculated peanuts ranged between 55.0 and 61.0% during the test period (Figs. 1-3). Lipid content of whole unfractionated ground control peanuts ranged from 44.0 to 47.0%, while buffer soluble extracts contained between 5.0 and 12.0% ether extractable material during the 18-day trials. In general, percentage ether soluble material in combined fat pad-pellet preparations of peanuts inoculated with the different fungi declined as the test period progressed to day 18. Simultaneously, percentages of amber-colored constituents remaining with the aqueous extract increased quantitatively.

Although similarities were noted in the percentage dis-

tribution of ether extractable material in buffer soluble and insoluble fractions of peanuts infected with the four fungi, characteristic differences typified each organism. Of the four fungi, *A. parasiticus* caused the greatest changes in percentage ether soluble material among fractions (Fig. 1). Diethyl ether extractable material in the aqueous fraction of infected peanuts was 6- to 12-fold greater than that of uninoculated peanuts between 4 and 18 days after inoculation. Simultaneously, levels of lipid in the buffer insoluble fraction decreased ca. 30.0%. A decrease in percentage lipids was noted in whole seeds infected with *A. parasiticus.* Percentage ether soluble material in the insoluble fraction of *A. parasiticus* mycelia/conidia was low and remained relatively constant during the 18-day test period. Levels of ether soluble material in the buffer soluble extract decreased by about half during this same time interval. Similar observations were made on buffer extracts of mycelia/conidia or spores of other test fungi included in this study (Figs. 1-3).

Data from experiments using *A. oryzae* are shown in Figure 2. Although percentage ether extractable material in the aqueous fractions of infected seeds increased during the test period, changes were not as great as those observed for *A. parasiticus.* Initial decreases in percentage ether soluble material in the biffer insoluble fraction were followed by increases during the later stages of the test period. Small increases in content of ether extractable material were noted in infected whole seeds as the test period progressed to day 18.

Figure 3 shows changes in percentage ether soluble material in whole seeds and buffer soluble and insoluble fractions of peanuts infected with *R. oligosporus* and *N. sitophila.* Changes in percentage ether extractable material in buffer soluble and insoluble fractions of infected seeds were similar to those noted for the aspergilli, especially *A. parasiticus*; i.e., percentage ether soluble material increased in aqueous extracts and decreased in insoluble preparations as fungal growth progressed. Although *N. sitophila* grew somewhat slower than the other test fungi, the rates of changes in percentage ether extractable material in various fractions were comparable.

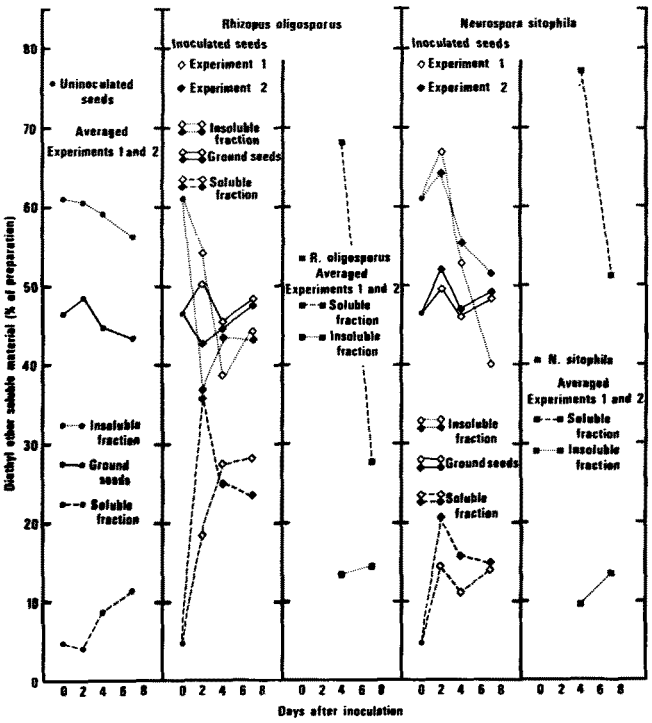

FIG. 3. Percentage diethyl ether soluble material in lyophilized ground seeds and buffer soluble and insoluble extracts of seeds and fungal growth (mycelia/conidia or spores). Seeds were not inoculated or inoculated with *Rhizopus oligosporus* or *Neurosporus sitophila* for various time intervals to day 7. Fungal material used in these analyses was collected from the surface of infected seeds.

DISCUSSION

Increases in levels of lipid in aqueous soluble extracts of infected peanuts undoubtedly resulted from lipolytic activity of the test fungi. The same strains of *A. oryzae, R. oligosporus,* and *N. sitophila* have been shown to hydrolyze peanut lipids to yield 16.3, 31.5, and 9.4% FFA, respectively, within a 4 day test period (20). Increases in FFA of autoclaved peanuts inoculated with aspergilli belonging to other taxonomic groups and with *Penicillium citrinum* after 2 weeks of infection have been reported (24). These researchers reported decreases in total oil after extended infection periods. The peanuts were not examined until after 2 weeks of infection. Others have studied *Rhizopus* solubilization of soybean oil (18) and lipase production by aspergilli on peanuts (25) and coconuts (26). The data presented in this paper show that it becomes increasingly difficult to prepare aqueous protein extracts containing low amounts of lipid components as fungal infection progresses in peanuts. It is assumed that much of the oil that is being hydrolyzed (21-23) is partitioning into aqueous extracts of infected peanuts. Based on previous studies, only a small portion of the lipid designated as ether extractable material in this study is probably nonlipid (20).

Techniques for the large scale extraction and concentration of protein from peanuts for food have, for the most part, been developed using high quality seeds. Data presented from the present study using infected peanuts, in addition to information available on fungal solubilization of oils from other vegetable materials (24-26), indicate that aqueous extraction procedures may require modification if suitable protein extraction is to be achieved. It is possible that extensive partitioning of lipid components of infected peanuts into aqueous extracts presently being evaluated for fractionating proteins may result in undesirable physicochemical and organoleptic properties in concentrates and isolates. We have shown that new problems may exist for the processor who intends to use aqueous extraction procedures for both infected and high quality peanuts.

In light of the shift in lipid distribution in peanut extracts as a result of infection, consideration might also be given to extraction characteristics of aflatoxins and other mycotoxins naturally occurring in infected peanuts. As a result of numerous degradation processes which occur as aflatoxigenic aspergilli invade peanuts, distribution of mycotoxins in various fractions may be quite different from that reported from studies involving external addition of known quantities of these toxins to high quality peanuts and meals prior to extraction (10).

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REFERENCES

1. Harris, H., E.Y. Davis, M.S. Van de Mark, K.S. Rymal, and J.J. Spadaro, Auburn Univ. Agric. Exp. Stn. Bull. 431 (1972).
2. Rhee, K.C., C.M. Cater, and K.F. Mattil, J. Food Sci. 37:90 (1972).
3. Mattil, K.F., Food Prod. Dev. 7(6):40 (1973).
4. Ayres, J.L., L.L. Branscomb, and G.M. Rogers, JAOCS 51:133 (1974).
5. Cater, C.M., K.C. Rhee, R.D. Hagenmaier, and K.F. Mattil, Ibid. 51:137 (1974).
6. Goldblatt, L.A., Ibid. 48:605 (1971).
7. Mann, G.E., H.E. Gardner, Jr., A.N. Booth, and M.R. Gumbmann, J. Agric. Food Chem. 19:1155 (1971).

8. Gardner, Jr., H.K., S.P. Koltun, F.G. Dollear, and E.T. Rayner, JAOCS 48:70 (1971).
9. McKinney, J.D., G.C. Cavanagh, J.T. Bell, A.S. Haversland, D.M. Nelson, J. Pearson, and R.J. Selkirk, Ibid. 50:79 (1973).
10. Natarajan, K.R., K.C. Rhee, C.M. Cater, and K.F. Mattil, Ibid. 52:44 (1975).
11. Cherry, J.P., L.R. Beuchat, and C.T. Young, J. Agric. Food Chem. 24:79 (1976).
12. Cherry, J.P., C.T. Young, and L.R. Beuchat, Can. J. Bot. 53:2639 (1975).
13. Cherry, J.P., R.Y. Mayne, and R.L. Ory, Physiol. Plant Pathol. 4:425 (1974).
14. Cherry, J.P., R.L. Ory, and R.Y. Mayne, J. Am. Peanut Res. Ed. Assoc. 4:32 (1972).
15. Wagenknecht, A.C., L.R. Mattick, L.M. Lewin, D.B. Hand and K.H. Steinkraus, J. Food Sci. 26:373 (1961).
16. Steinkraus, K.H., C.Y. Lee, and P.A. Buck, Food Technol. (London) 19:1301 (1965).
17. van Veen, A.G., D.C.W. Graham, and K.H. Steinkraus, Cereal Sci. Today 13:96 (1968).
18. Van Buren, J.P., L.R. Hackler, and K.H. Steinkraus, Cereal Chem. 49:208 (1972).
19. Yoshida, H., and G. Kajimoto, J. Jpn. Soc. Food Nutr. 25:415 (1972).
20. Beuchat, L.R., and R.E. Worthington, J. Agric. Food Chem. 22:509 (1974).
21. Diener, U.L., and N.D. Davis, Trop. Sci. 10:22 (1968).
22. Sanders, T.H., N.D. Davis, and U.L. Diener, JAOCS 45:683 (1968).
23. Landers, K.E., N.D. Davis, and U.L. Diener, Phytopathology 57:1087 (1967).
24. Ward, H.S., Jr., and U.L. Diener, Ibid. 51:244 (1961).
25. Ramakrishnan, C.V., and B.N. Banerjee, JAOCS 29:596 (1952).
26. Hoover, R., S.F. Laurentius, and K.G. Gunetileke, Ibid. 59:64 (1973).

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