number of metabolites: three in the mouse, two in the rat, and one in the house fly. They are designated A, B, C (metabolites), and D (photodieldrin). The R_f values of A, B, C, and D are 0.27, 0.36, 0.43, and 0.48, respectively, in benzene–ethyl acetate (3:1) solvent system. The metabolites on tlc plates, corresponding to the darkened areas of the X-ray film, were scraped off and extracted with acetone for liquid scintillation counting and glc analysis. The per cent recoveries of photodieldrin or its metabolites are given in Figure 1. Our attempts to detect these extracted metabolites by glc using various columns (6% DC-200 on Varaport-30, 5% QF-1 and 3% SE-30 or SE-52 on Chromosorb W) were not successful. The reason for nondetectability on glc may be due to either low levels or nonvolatile nature or nonelution of the compounds under these conditions. Similar observations have been reported in the case of [^{14}C]photodieldrin metabolites *in vivo* (Klein *et al.*, 1970).

That these metabolites are enzymatic products of the mixed-function oxidase was confirmed by the lack of metabolism in the absence of NADP or inhibition of the metabolism in the presence of 10 μg of piperonyl butoxide (an inhibitor of MFO) in the incubation.

There were no qualitative differences in the metabolic products formed between male and female mice. However, only the male and not the female rat showed the degradation of photodieldrin. This may be related with higher levels of MFO activity in the male rat. For example, the specific activities (picomoles/microgram of protein per minute) of epoxidation of aldrin and photoaldrin were, respectively, 3.6 and 4.2 by male and 0.5 and 0.8 by female rat MFO. Such differences in MFO activity toward cyclodienes between male and female rats have been reported by other workers (Wong and Terriere, 1965). Differences between male and female rats in the *in vivo* metabolism, distribution, and excretion of photodieldrin have also been observed by several workers (Klein *et al.*, 1970; Dailey *et al.*, 1970, 1972).

The present investigation thus provides evidence that photodieldrin is oxidatively metabolized to lipophilic products by rat, mouse, and house fly mixed-function oxidase. The chemical nature of these metabolites and their toxicity should be investigated to understand the significance of this metabolic conversion.

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Alkylthiazoles in Potato Products

2,4,5-Trimethylthiazole and 2-isopropyl-4,5-dimethylthiazole have been characterized in the basic fraction of the volatile oil of potato chips using the combination of capillary gas chromatography and mass spectrometry. The above compounds and the additional compounds 2-isopropyl-4-methyl-5-ethylthiazole, 2-isobutyl-4,5-dimethylthiazole, and 2-acetylthiazole have also been characterized in the basic fraction of the volatile oil from boiled potatoes.

A number of alkyl- and alkanoylthiazoles have been found in foods, particularly roasted foods. Those found up to the present are summarized in Table I. Other related compounds that have been found in foods are 2-acetylthiazoline (Tonsbeek *et al.*, 1971) in beef broth and benzothiazole in a great variety of foods.

In the authors' studies of the volatile components of potato products over the last several years a number of alkylthiazoles have been detected. Because of the potent and characteristic odor of some alkylthiazoles these compounds could be important to the aroma and flavor of potato products.

Table I. Alkyl- and Alkanoylthiazoles Previously Reported in Cooked Foods

Compound	Food
Thiazole	Roasted peanuts,[a] cooked beef[b]
2-Methylthiazole	Cooked beef[b]
4-Methylthiazole	Roasted peanuts,[a] cooked beef[b]
2,4-Dimethylthiazole	Cooked beef[b]
4-Methyl-5-ethylthiazole	Cooked beef[b]
2-Methyl-4-ethylthiazole	Cooked beef[b]
2,4,5-Trimethylthiazole	Cooked beef[b]
2,4-Dimethyl-5-vinylthiazole	Cooked beef[b]
4-Methyl-5-vinylthiazole	Roasted filberts[c]
2-Acetylthiazole	Cooked beef[b]
2-Acetyl-4-methylthiazole	Roasted coffee[d]
2-Propionyl-4-methylthiazole	Roasted coffee[d]

[a] Wàlradt *et al.*, 1971. [b] Wilson *et al.*, 1973. [c] Kinlin *et al.*, 1972. [d] Stoll *et al.*, 1967.

EXPERIMENTAL SECTION

Materials. Good quality potato chips were obtained from a local retail market. Oregon Russet Burbank potatoes were obtained from a local wholesale market.

Alkylthiazoles were synthesized using methods outlined previously (Buttery *et al.*, 1973). Structures were checked by pmr spectra.

Isolation of Volatile Basic Fractions. The basic fraction of the volatile oil of potato chips was obtained as described previously (Buttery *et al.*, 1971). The basic fraction from the volatile oil of boiled potatoes was obtained essentially as described previously for raw potatoes (Buttery and Ling, 1973), except that the steam distillation continuous extraction was carried out at atmospheric pressure instead of vacuum.

Gas–Liquid Chromatography (Glc)–Mass Spectral Analysis. For the potato chip volatile basic fraction the glc column used was 300 m × 0.075 cm i.d. stainless steel capillary coated with Silicone SF96(100) containing 5% Igepal CO-880.

For the boiled potato basic fraction the glc column was a 150 m × 0.075 cm i.d. stainless steel capillary coated with Amine 220 containing 5% Igepal CO-880.

For each study the capillary column was coupled directly to a Consolidated 21-620 cycloidal type mass spectrometer through a silicone membrane molecular separator.

RESULTS AND DISCUSSION

As outlined in a previous paper (Buttery *et al.*, 1971), most of the components of the basic fraction of the volatile oil of potato chips are alkylpyrazines. There were, however, a few components with an odd numbered molecular weight indicating compounds containing only one nitrogen (or an odd number) in the molecule. As other features of the mass spectra also indicated the presence of sulfur, these components were suspected to be alkylthiazoles. The identification of one of these and of related compounds in other products (Table I) and further work by the authors on the mass spectra of alkylthiazoles (Buttery *et al.*, 1973) assisted considerably in the characterization of the compounds in the present work.

The compounds characterized in the basic fraction from the volatile oil of potato chips are shown in Table II. These alkylthiazoles occurred at about the same order of concentration as that of many of the minor alkylpyrazines in potato chips, but were of the order of one-tenth the concentration of the major alkylpyrazines. Other alkylthiazoles may be present also in the potato chips in smaller amounts, but are obscured by the abundant alkylpyrazine components.

Table II. Alkyl- and Alkanoylthiazoles Characterized in the Basic Fractions from the Volatile Oils of Potato Chips and Boiled Potatoes

Confirmed identity[a]	
Potato chips	Boiled potato
2,4,5-Trimethylthiazole, MS, RT[b]	2,4,5-Trimethylthiazole, MS, RT
2-Isopropyl-4,5-dimethylthiazole, MS, RT	2-Isopropyl-4,5-dimethylthiazole, MS, RT
	2-Isopropyl-4-methyl-5-ethylthiazole, MS, RT
	2-Isobutyl-4,5-dimethylthiazole, MS, RT
	2-Acetylthiazole, MS, RT
	Tentative identity[c]
	Dipropylthiazole, MS
	Ethyldimethylthiazole, MS

[a] Evidence cited consistent with that of an authentic sample. [b] MS, RT = mass spectral and glc retention evidence, respectively. [c] Spectra similar but apparently of mixtures.

Several additional thiazoles were detected in the basic fraction of the volatile oil of boiled potatoes. Those characterized are listed in Table II. The concentration of these in the boiled potatoes was calculated to be rather low, less than about 1 part in 10^9. There was considerable variation with different lots of potatoes. Many lots of potatoes did not contain any detectable amount of thiazoles, due either to the extremely small amounts or to being obscured by other components. We also know very little about the efficiency of isolation of such small concentrations of such compounds.

Besides the alkylthiazoles mentioned in Table II, a number of compounds with related mass spectral patterns were detected. These seem to be different variations of the possible alkyl substituted thiazoles. There is apparently almost the complexity that is found with the alkylpyrazines occurring in foods, and a related mechanism of formation may be involved (*cf.* Schutte, 1974).

2,4,5-Trimethylthiazole and 2-acetylthiazole have been reported as occurring in beef volatiles (Wilson *et al.*, 1973). However, as far as the authors can determine, the other compounds in Table II have not been previously found in nature. 2-Isopropyl-4-methyl-5-ethylthiazole had been previously synthesized by Theil *et al.* (1961). We have not been able to find previous reference to 2-isopropyl-4,5-dimethylthiazole and 2-isobutyl-4,5-dimethylthiazole in the literature although many related alkylthiazoles have been synthesized over the last 10 years by Metzger and coworkers (*cf.* Roussel *et al.*, 1971).

The mass spectra of the compounds previously unreported in nature are listed below (two most intense ions every 14 mass units above *m/e* 34; intensities in parentheses; molecular ion in italics): 2-isopropyl-4,5-dimethylthiazole, 39 (20), 45 (30); 53 (21), 59 (23); 70 (6), 71 (39); 86 (26), 87 (16); 96 (17), 97 (2); 113 (11), 114 (6); 125 (2), 127 (2.4); 140 (100), 141 (11); 154 (12), *155* (40); 2-isopropyl-4-methyl-5-ethylthiazole, 41 (37), 45 (45); 55 (13), 59 (26); 67 (12), 71 (19); 85 (27), 87 (5); 96 (18), 100 (15); 112 (2), 113 (1); 121 (3), 127 (9); 139 (16), 140 (13); 154 (100), 155 (20); 168 (10), *169* (42); 2-isobutyl-4,5-dimethylthiazole, 41 (15), 45 (14); 53 (8), 59 (13); 68 (2), 71 (18); 85 (9), 86 (22); 94 (0.2), 99 (0.4); 126 (33), 127 (100); 154 (12); 168 (4), *169* (10).

With the tentatively characterized compounds the mass spectra of the compounds obtained from potatoes were

clearly mixtures. The mass spectra of authentic ethyldimethylthiazoles and 2,4- and 2,5-dipropylthiazole were reported previously (Buttery *et al.*, 1973).

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Effects of Freezing in the Presence of β-Mercaptoethanol on the Antigenicity of Peanut Proteins

Using immunoelectrophoresis, qualitative effects of freezing in the presence of β-mercaptoethanol (R-SH) on the immunological properties of peanut proteins were determined. When total protein extracts were frozen, only three major proteins remained soluble and antigenic after thawing. Isolated proteins of the arachin and conarachin systems were completely inactivated after freezing in R-SH; the conarachin proteins were more sensitive to reduction than arachin. Slight heterogeneity in migrations was observed for some of the proteins after treatment with R-SH. These results suggested that some determinant groups could be maintained by intra- and/or interchain disulfide bonds.

Thiol compounds are widely used as protective reagents for sulfhydryl groups, enzyme activators, and as reducing agents for disulfides. Depending on the stoichiometry and equilibrium constants for a given system, effects of these reagents vary with concentration and experimental conditions (Wall, 1971). In biological studies of plant and animal tissues, samples are often frozen awaiting experimentation. Changes in electrophoretic migrations and enzyme activities of peanut proteins as effected by thiol reagents and freezing have been reported (Cherry and Ory, 1973). Characterization of the major peanut proteins by immunoelectrophoresis (IEA) was reported previously (Daussant *et al.*, 1969). The purpose of this study was to determine the effects on antigenic properties of peanut globulins (total extracts and isolated fractions) after they were treated with β-mercaptoethanol (R-SH) and then frozen and thawed.

EXPERIMENTAL SECTION

Whole (full-fat) seeds were extracted in phosphate buffer (pH 7.9, ionic strength 0.01) and centrifuged, and the supernatants were made to 0.02 or 0.25 *M* R-SH and frozen for 15 hr (including a control) before analysis. Isolated α-arachin (Neucere, 1969) and the conarachins (Dechary *et al.*, 1961) were made to 0.02 or 0.25 *M* R-SH in phosphate buffer for similar analyses.

Protein contents were determined by the method of Lowry *et al.* (1951). Immunoelectrophoresis was performed in Ionagar No. 2 (Colab Laboratories, Inc., Glenwood, Ill.) according to Grabar and Williams (1953) employing 4 V/cm for 2 hr at room temperature. All sample wells were filled with approximately 0.75 mg of protein and all troughs were filled three times with immune serum *vs.* the fresh total protein extract. After washing out serum proteins, the precipitin lines were stained with 0.1% Amido Black in 7.0% acetic acid and destained with 7.0% acetic acid. The immune serum against the total protein extract was prepared by Antibodies, Incorporated (Davis, Calif.).

RESULTS AND DISCUSSION

Figure 1 shows the IEA of total protein extracts in R-SH after freezing and thawing. No major changes were observed in the total precipitin patterns before freezing (compare the first three samples at the top). A slight cathodic shift of components A and B was observed in the samples containing R-SH, however. After freezing in 0.02 and 0.25 *M* R-SH, only the supernatant of 0.25 *M* R-SH showed a major change in pattern. In the latter, only some subunits of α-arachin (D), one of the so-called α-arachin contaminants (A), and α_2-conarachin (E) retained their immunological properties. The corresponding precipitates shown in the last three samples were very similar except one of the components (F) was not observed in the 0.25 *M* R-SH precipitate.

To test the effect of the reagent on isolated protein, purified α-arachin and the conarachin fraction were treated with R-SH and tested before and after freezing. Since one end of the molecule is an alcohol that could possibly cause denaturation without effecting disulfide bonds, an additional control treated with 0.25 *M* ethanol was used. These results are shown in Figure 2. For the α-arachin preparation (part A) no changes in antigenicity were induced by either 0.02 *M* R-SH or ethanol before and after freezing. After freezing in 0.25 *M* R-SH, no antigenicity for α-arachin was observed in either the supernatant or