The volatile compounds were isolated from 540 lb of baked Russet Burbank potatoes. After extensive gas chromatographic fractionation, the relative pure fractions were analyzed by GC–mass and infrared spectrometry.

The eight major mass fragmentation ions of each halogenated compounds identified are listed in Table I.

Since most of the halogenated compounds analyzed did not have major functional group peaks in their IR spectra, the fingerprint matching identification technique was used.

## RESULTS AND DISCUSSION

Fourteen halogenated compounds identified in the volatile compounds of baked potatoes are listed in Table I. The eight major mass fragmentation ions of each compound are also listed in Table I. The mass spectra of these compounds are in good agreement with the published data (Eight Peak Index of Mass Spectra, 1974).

There are two possibilities for the formation of these halogen compounds which were identified in baked potato. The first is the persistence of now banned insecticides in established turf. Since 1943, many chlorinated hydrocarbon insecticides have been used including DDT, toxaphene, chlordane, dieldrin, aldrin, and heptachlor. These chlorinated hydrocarbons are not very volatile and are persistent in the soil (Brown, 1978). Second, some halogenated insecticides and fungicides are still in use on potatoes used for baking. The decomposition of pesticide residues or the interaction of pesticide residues with potato during baking may lead to the formation of the halogenated compounds identified.

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# Trypsin-Inhibitor and Chymotrypsin-Inhibitor Studies with Soybean Extracts

The effect of dry and wet heat treatments on proteinase inhibitors of soybean flour was studied. The inhibitors were extracted from the flour by physiological saline before or after the heat treatment. The inhibitors showed a higher heat resistance in the dry flour than after extraction by saline. The heat inactivation curve of trypsin- and chymotrypsin-inhibiting capacities differed more in the case of the wet treatment. It was also found that trypsin and $\alpha$-chymotrypsin could release themselves from the inhibition to some extent on standing for long periods.

Raw soybean flour contains several antinutritive substances, among others proteinase inhibitors. The latter is composed of two major fractions: the inhibitors of Kunitz (1945) and of Bowman and Birk (Bowman, 1946; Birk et al., 1963). The former inhibits trypsin well and $\alpha$-chymotrypsin slightly, while the latter is effective on both enzymes.

In the present experiments the total trypsin- and chymotrypsin-inhibiting capacities of soybean flour were compared under different modes of heat inactivation. The effect of incubation time on the proteinase–soybean inhibitor system was also studied.

## EXPERIMENTAL SECTION

**Preparation of Samples.** Fat-containing soybean flour with 93% dry matter was used throughout. Samples (0.5 g) of flour were weighed into Erlenmeyer flasks, and 24.5 mL of physiological saline was added. The flasks stood at room temperature for 4 h and were shaken periodically by hand. Then they were kept in a refrigerator at 4 °C overnight. The next day the samples were shaken again periodically at room temperature for 1 h, centrifuged, and frozen until used. In experiment 1, heated soybean flour samples were extracted; in experiment 2 and 3 unheated

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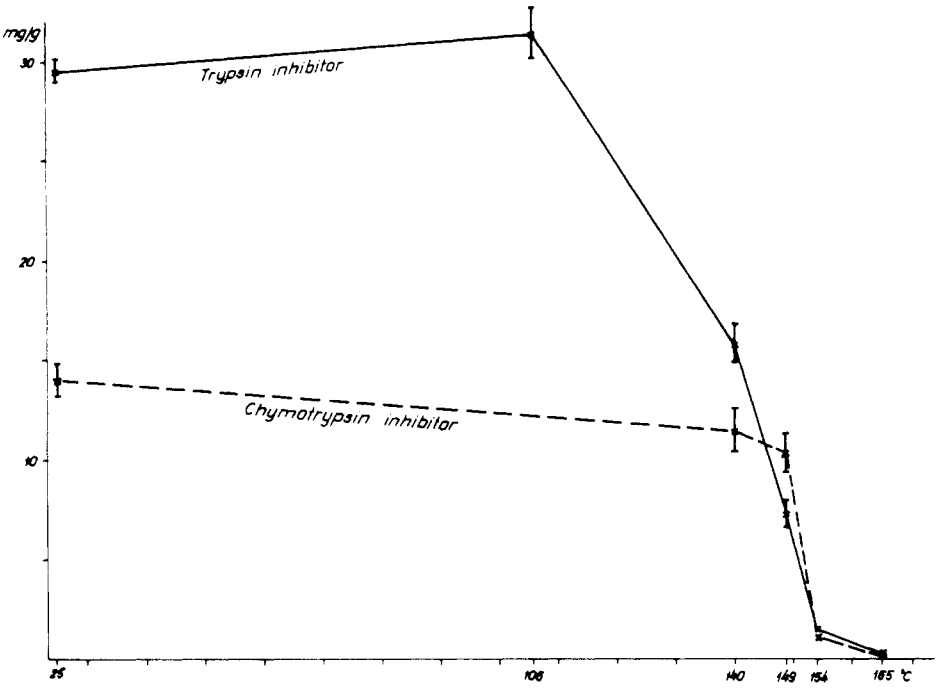


**Figure 1.** Trypsin-inhibiting and chymotrypsin-inhibiting capacities of soybean flour samples heated at different temperatures for 1 h. Values represent amounts of proteinase (in milligrams) inhibited by 1 g of soybean flour (93% d.m.). Mean of five samples ± standard error of the mean.

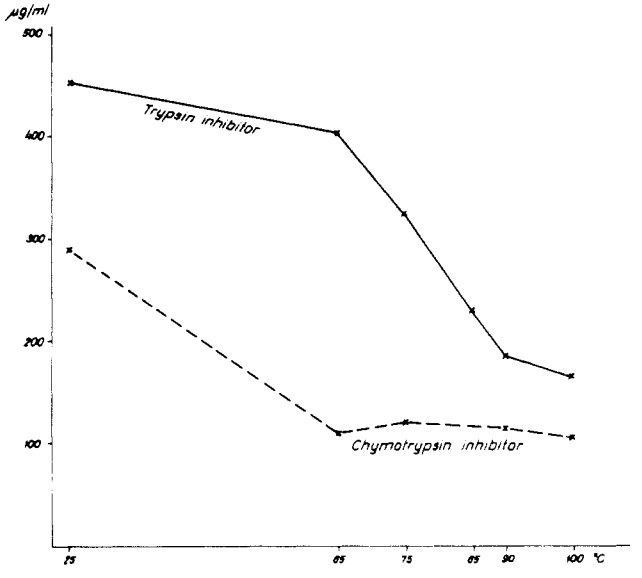


**Figure 2.** Trypsin-inhibiting and chymotrypsin-inhibiting capacities of saline extracts of soybean flour. Extracts were heated at different temperatures for 10 min. Values represent micrograms of proteinase inhibited by 1 mL of soybean flour extract.

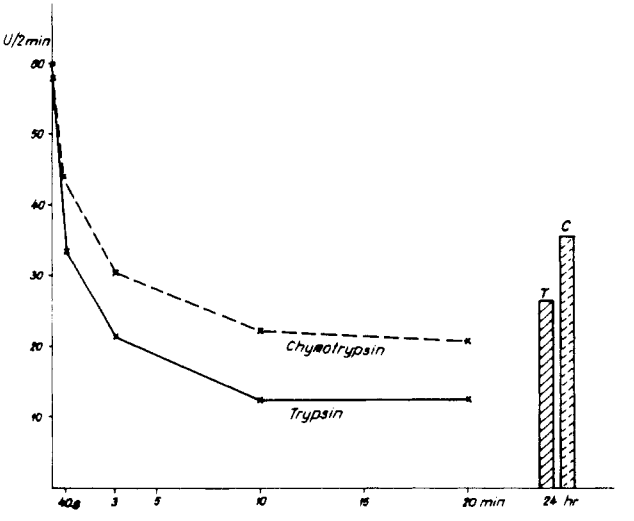


**Figure 3.** Inhibition of tryptic and chymotryptic activities by a saline extract of soybean flour. The columns indicate the same activities after 24-h incubation at room temperature. U = 0.001 increase of extinction; T = trypsin; C = chymotrypsin.

ones were extracted.

In experiment 1, each sample was heated 1 h at different temperatures, in an oven. In experiment 2, samples of an extract of unheated soybean flour were incubated in a water bath at different temperatures for 10 min.

**The inhibitors were determined** by measuring the decrease in activity of a known amount of respective protease in the presence of sample. The results were calculated from approximately 30–50% (trypsin) or 20–40% (chymotrypsin) inhibition. Proteolytic activities were determined based on the principle of Schwert and Takenaka (1955) in pH 8 Tris-HCl buffer at room temperature. The substrate of trypsin was benzoylarginine ethyl ester (BAEE; 253 nm) and for α-chymotrypsin benzoyltyrosine ethyl ester (BTEE; 256 nm). The results were expressed in milligrams of inhibited proteinase (experiments 1 and 2) or in BAEE and BTEE units (experiment 3).

## RESULTS AND DISCUSSION

**Experiment 1.** Trypsin- and chymotrypsin-inhibiting capacities of heated soybean flour samples are shown in Figure 1. In the case of unheated control samples, the chymotrypsin inhibitor values approximated half of the trypsin inhibitor values. This may be due to the fact that trypsin is inhibited by the Kunitz and the Bowman–Birk inhibitors whereas the Kunitz inhibitor contributes little to the total chymotrypsin-inhibitor capacity. The chymotrypsin inhibitor began to be inactivated later than the trypsin inhibitor. The trypsin-inhibiting capacity first declined to the level of the chymotrypsin inhibitor, and then above ~145 °C their inactivation paralleled each

other. At 165 °C the inhibitors have practically disappeared. The flour turned brown in the same temperature range where the inhibitors were inactivated. This parallelism suggests that the dry heat inactivation is at least partially due to the Maillard reaction.

**Experiment 2.** The changes in trypsin- and chymotrypsin-inhibitor activities after heating the saline extract of soybean flour are shown in Figure 2. The unheated control extract inhibited approximately twice as much trypsin as chymotrypsin, similar to that in the previous experiment. After the extract was kept at 65 °C for 10 min, protein precipitation was noticed, which was removed by centrifugation. No significant amounts of active inhibitor were regained from the precipitate. At this temperature the chymotrypsin-inhibiting capacity fell steeply, while the trypsin inhibitor did the same though less markedly. Between 65 and 100 °C the chymotrypsin inhibitor did not decrease further, while the trypsin inhibitor declined to the greatest extent in this temperature range.

Comparing the effects of dry and wet heat treatment, it is noteworthy that the inhibitors have a much higher resistance in dry environment than after extraction. In each treatment the most heat-stable fraction inhibited both trypsin and chymotrypsin. This fraction is probably identical with the Bowman–Birk inhibitor, which has a higher heat stability than the Kunitz inhibitor even in purified state. Inactivation of the latter seems to occur in those temperature ranges where only a decrease of trypsin inhibitor is observable.

**Experiment 3.** The progress of the reaction of trypsin or chymotrypsin with the saline extract of soybean flour is shown in Figure 3. The decline of activity of both proteinases showed similar curves. The reaction was completed in ~10 minutes at room temperature. However, when the solutions stood overnight, the activity of proteinases increases again. This indicates that both trypsin and chymotrypsin can digest a part or some fractions of the inhibitors during longer periods of incubation.

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# Changes in Chlorogenic, Caffeic, and Quinic Acid Contents during Sunflower Seed Maturation

Changes in chlorogenic (CGA), caffeic (CA), and quinic (QA) acid contents in sunflower seeds (EC 68415) were determined during seed maturation. CGA content increased slowly from 21 to 28 days after flowering, rapidly from 28 to 35 days, and again slowly up to 43 days when the CGA content was maximum. CA decreased appreciably and QA slightly from 21 to 28 days, and thereafter CA decreased slowly and QA rapidly up to 43 days. From then until 49 days, CGA decreased to a small extent while CA and QA increased appreciably. Variations in CGA, and CA, and QA during seed maturation thus appear to be interrelated.

The presence of chlorogenic acid (CGA) as well as its components, caffeic (CA) and quinic (QA) acids, in sunflower seeds is well-known (Sechet et al., 1959; Milic et al., 1968). The presence of CGA is not desirable since it causes discoloration of proteins at alkaline pH (Smith and Johnsen, 1948). CGA is reported to vary with the position of seed in the flower head due to differences in the physiological state of maturity (Pomenta and Burns, 1971) and with cultivar (Brummet and Burns, 1972). However, information on variations in the contents of CGA as well as CA and QA with seed maturation is lacking, and results of such an investigation are reported here.

## MATERIALS AND METHODS

Samples of sunflower seeds *Helianthus annuus* L. (var. EC 68415) were collected from plants grown on a bulk plot in the fields of Hayathnagar Research Farm (ICAR), Hyderabad, at different stages of seed maturation from 21 to 49 days after flowering, which occurred 60 days after planting. A composite seed sample (~40 g) was prepared by mixing all the seeds from the flower heads of 20 plants selected at random. The seeds were decorticated, and oil was extracted from the kernels with *n*-hexane in a Soxhlet apparatus. The defatted meal was desolventized by spreading in a thin layer and exposing to the atmosphere for 8 h at room temperature (30 °C). Standard procedures used for the analysis of sunflower seed deoiled meals, namely, those of Sosulski et al. (1972) for CGA and CA contents and of Mesnard and Devaux (1964) for QA content, were followed. Absorption was measured at 324 and 320 nm for CGA and CA, respectively (Cater et al., 1972). Standard curves were prepared for CGA and CA (Cater et al., 1972) and for QA (Mesnard and Devaux, 1964) from chemicals (over 96% pure) purchased from Sigma Chemical Co., St. Louis, MO, USA after drying them overnight at 50 °C under vacuum. The purity of these chemicals was determined by comparison with pure samples isolated by preparative thin-layer chromatography on 1 mm thick silica gel G using butanol–acetic acid–water (4:1:5 v/v).

## RESULTS AND DISCUSSION

The relative changes in contents of CGA, CA, and QA in the defatted meal samples from seeds at different stages of maturation are given in Table I. The data show that the CGA content increased with progressive maturation of the seed, gradually from 21 to 28 days after flowering,

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