

icance.

All these components were positively identified by GC-MS, but since mass spectra of some of these esters have not been widely published, Table III provides summaries of the spectra of the less common compounds.

From a biosynthetic point of view the presence of two such similar, very closely related series of compounds is most intriguing, and presumably compounds within a series must be associated in some way, although this has not been suggested before. Heinz and Jennings (1966) have, however, pointed out that even carbon number carboxylic esters and their hydroxy derivatives could arise from condensation reactions involving acetyl-CoA and malonyl-CoA, but no involvement of related 2-enoates has been suggested. However, in the series reported here the double bond and the hydroxyl group are attached to the same carbon atom of the parent acid, so clearly the 2-enoates could be condensation products of the 3-hydroxy esters, or possibly the sequence could be reversed. It is also most interesting to find the parent acid and alcohol present in the same system.

The β -hydroxy esters have been reported as flavor components before, but not frequently, and they are very much less common aroma components than the parent esters. Nevertheless, certain members have been identified in pineapple (Creveling et al., 1968), passion fruit (Murray et al., 1972; Winter and Kloti, 1972), orange, grapefruit (Moshonas and Shaw, 1971), tangerine (Moshonas and Shaw, 1972), and grapes (Stern et al., 1967), although the full range found in wood apple has not been determined elsewhere. The 2-enoates are less common still, but ethyl but-2-enoate and ethyl hex-2-enoate have been found in passion fruit (Murray et al., 1972; Winter and Kloti, 1972) and grapes (Stern et al., 1967). What is immediately ob-

vious and highly intriguing about this list of previous reports of these esters is that all apply to tropical or subtropical fruits. The results described here for wood apple augment this compilation, and it may even be that these particular esters might prove characteristic of a particular type of tropical fruit flavor.

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Extractability of Nitrogenous Constituents from Iraqi Mung Bean As Affected by pH, Salt Type, and Other Factors

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Factors influencing nitrogen extractability (pH, temperature, time, and flour to solvent ratio) were studied extensively, and the extraction profile was determined with every variable when keeping other variables constant. In addition, the effects of seven salts in wide ranges of concentrations on nitrogen dispersibility were investigated. The maximum nitrogen extractabilities attained by these salts, arranged on a decreasing order, are as follows: 87.58% with 0.100 N sodium carbonate, 82.22% with 0.025 N sodium sulfite, 79.93% with 0.050 N sodium citrate, 78.78% with 0.500 N sodium chloride, 78.40% with 0.250 N sodium oxalate, 74.95% with 0.010 N sodium acetate, and finally 74.80% with 100 mg/L Na₂EDTA compared with 79.00% with distilled water. Moreover, when the trends of the nitrogen extractability patterns obtained for the seven salts were compared, it was possible to divide them into three general types.

Nitrogen dispersibility for many legume seeds in aqueous solutions of hydrochloric acid and sodium hydroxide at different pH values was studied by numerous investigators (Evans and Kerr, 1963; Pusztai, 1965; Pant and Tulsiani, 1969; Hang et al., 1970; Fan and Sosulski, 1974;

Coffmann and Garcia, 1977; Thompson, 1977). Their results indicated that the highest nitrogen dispersibility occurred at pH 1.0-2.0 in the acidic range and above pH 7.0 in the basic range with minimum solubility occurring in the pH range 4.0-5.0. Moreover, Hang et al. (1970) concluded that the amount of nitrogen extracted from mung beans at alkaline pH was greater than that produced at their neutral or acidic pH values and the minimum point of nitrogen dispersion occurred at pH 4.0. However, recovery from soybeans and horsebeans was increased by increasing the temperature to 45 °C and by efficient

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shearing action of various stirrings (Circle and Smith, 1977; Patel and Johnson, 1974).

Longer extraction time gave slightly higher protein yield as extending extraction time from 5 to 15 min at 23 °C enhanced protein dispersibility from 84.4 to 95.8% of the total horsebean protein but no further solubilization occurred beyond 15 min up to 1 h. About 84% of the total protein was extracted within 5 min of agitation, indicating that the bulk of the protein was dispersed rapidly (Patel and Johnson, 1974).

Many laboratory studies indicated a 1:10 (meal/solvent) ratio to be adequate with or without a second extraction at a 1:15 ratio. A 1:20 or 1:40 ratio may remove a greater amount of the total protein but also results in a more dilute protein solution (Wolf, 1977). However, with horsebean flour, protein was solubilized best at a solvent ratio of 1:5 amounting to 88.3% and at a ratio of 1:10 amounting to 94% and less well at a ratio of 1:15 amounting to 88% (Patel and Johnson, 1974).

Dispersibility of nitrogenous constituents of finely ground legume pulses in aqueous salt solutions was investigated by many investigators (Pant and Tulsiani, 1969; Hang et al., 1970; Krishnamurthy et al., 1976). Different results were obtained due to the large number of factors involved. Gheyasuddin et al. (1970a) demonstrated that aqueous sodium phosphates extracted much less protein from sunflower meal than sodium chloride solutions and that extraction decreased with higher concentration. Moreover, sodium sulfite extracted more nitrogen at lower concentrations than at higher ones where extraction dropped drastically. Still, sodium sulfate was found to be a very poor solvent while sodium acetate gave a slight increase in protein extraction as the concentration of the salt increased.

Nevertheless, Hang et al. (1970) showed that dilute solutions of sodium chloride, sodium sulfate, calcium chloride, and magnesium chloride were found to have an inhibitory effect on the dispersion of the nitrogenous matter of mung beans, pea beans, and red kidney beans. However, alkaline salts such as sodium carbonate, disodium phosphate, and sodium citrate appeared to be fairly effective dispersing agents.

The objective of the present work was to study the extractability of nitrogenous constituents from Iraqi whole mung bean (IWMB) flour under wide ranges of pH, temperature, and extraction time as well as by using seven different salts in very wide ranges of concentrations.

EXPERIMENTAL SECTION

Mung bean seeds (*Phaseolus aureus*) were obtained from the Crop Science Department, College of Agriculture, University of Baghdad. It was grown in Abou Gharib fields in a suburb of Baghdad (Iraq).

Dirt and stones were removed, and the pulses were washed with water and dried to about its original moisture content (9.19%) by using sun drying. Samples were finally ground to flours by using a junior Brabender automatic laboratory mill (West Germany). The flour was sifted to pass a 0.63 mm diameter screen, and it had 24.95% protein, 0.96% crude fat, 3.42% ash, 4.36% crude fiber, and 66.32% carbohydrate, all given on dry weight basis.

Acid and Base Extractions. Extraction experiments were carried out on 5 g mung bean flour samples. Each was dispersed in 200 mL of alkaline (sodium hydroxide) or acidic (hydrochloric acid) solutions having the desired pH within the range 0.5–12.0. Several experiments were carried out to cover the whole range of pH with a 0.5 pH unit interval. Smaller pH unit intervals were maintained in the pH range of minimum solubility. The pH mea-

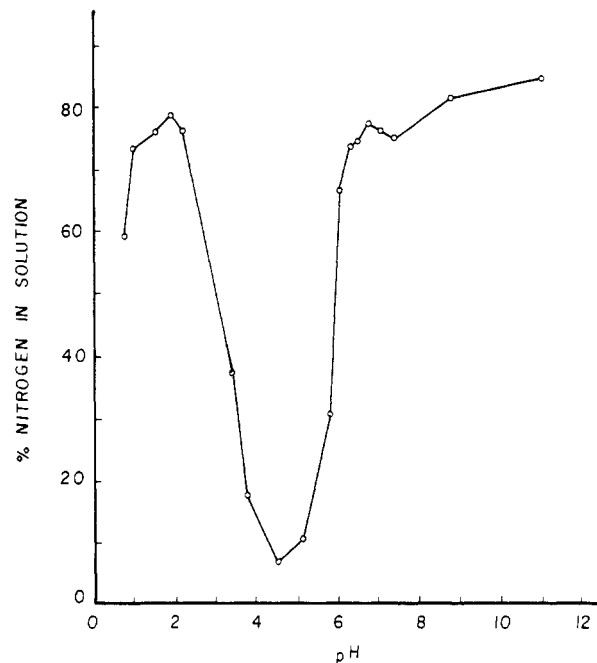


Figure 1. Extraction profile for Iraqi whole mung bean protein with pH measured for the protein extract.

surements were carried out for the final protein extract using a pye Unicam pH meter (9410 pH meter, Philips).

The mixture temperature was maintained at 25 °C, and extraction was performed by stirring with a magnetic stirrer (Ikacomimag RCH magnetic stirrer) for 15 min unless otherwise mentioned. The slurry was filtered under suction through a Whatman No. 4 filter paper, and the final pH of the extract was measured. A pH 7 was maintained constant while studying the effect of other extraction parameters on nitrogen extractability. Total nitrogen was determined in 5 mL of filtrate of each extraction experiment by using the standard macro-Kjeldahl method (Pearson, 1975).

Extraction times of 5, 10, 15, 20, 25, 30, and 60 min as well as flour/solvent (weight/volume) ratios of 1:5, 1:10, 1:15, 1:20, 1:30, and 1:40 were varied while other extraction parameters remained constant (at 25 °C, pH 7 for 15 min). The effect of temperature was examined in the range 25–60 °C. Duplicate extraction experiments were carried out in every case and mean nitrogen values were calculated.

Salt Extractions. Five grams of mung bean flour was mixed in 200 mL of a salt solution with a definite concentration. A wide range of salt concentrations (e.g., 0.005–2.000 N) were used with each of the salts; sodium sulfite, trisodium citrate, sodium oxalate, sodium acetate, sodium carbonate, and sodium chloride. Disodium ethylenediamine tetraacetate (Na_2EDTA) was used in the range from 0 to 1000 mg/L. Stirring was performed as mentioned earlier for 15 min at 25 °C. The slurry was filtered through Whatman No. 4 filter paper under suction.

Final pH values as well as nitrogen determinations in the filtrate were all carried out as mentioned earlier.

RESULTS AND DISCUSSION

Acid and Base Extractions. The effect of pH, temperature, time of extraction, and flour to solvent ratio on nitrogen extractability from IWMB flour was determined to establish the best conditions for nitrogen extraction. The effect of one parameter on the percentage of nitrogen extracted was determined while holding other parameters constant.

Effect of pH. Figure 1 shows nitrogen dispersibility from IWMB flour vs. pH of the protein extract. It indicated

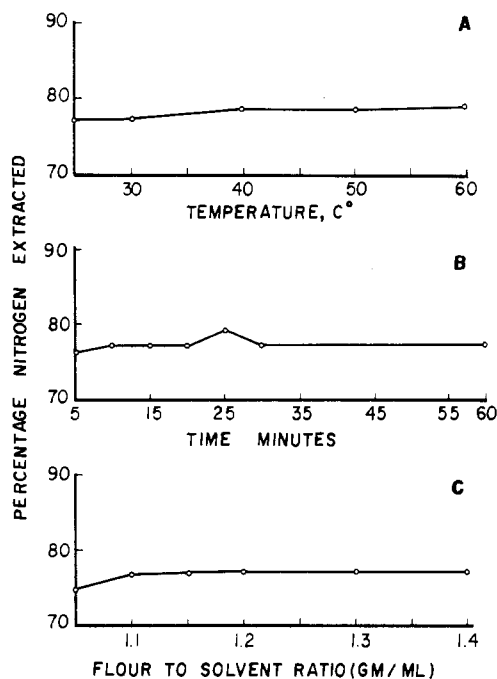


Figure 2. Effect of temperature (A), time (B), and flour to solvent ratio (C) on the extraction of nitrogen from Iraqi whole mung bean flour.

that dispersibility was higher at alkaline pH values than at either neutral or acid pH values. A region of low nitrogen extractabilities was observed between the final pH values of 3.4 and 5.7 when only 6.88–37.10% of nitrogen was extracted. However, the minimum extractability of nitrogenous constituents occurred at the pH of 4.5 (6.88%), and it remained low up to pH 5.20 when only 10.17% of nitrogen was extracted. The data of Figure 1 therefore suggested that most IWMB flour proteins have their isoelectric points through the pH range of 3.85 up to 5.2. These findings compared favorably with data given by some investigators. Fan and Sosulski (1974) reported minimum extractability of nitrogenous constituents from mung bean flour to be within the range of 4–5. Furthermore, Hang et al. (1970) concluded that several bean proteins, namely, mung bean, pea bean, and red kidney bean, had a common point of minimum dispersion at pH 4.0. The low protein extractabilities at pH values of 4.0–6.0 were essentially attributed to the intermolecular attraction of protein molecules in the isoelectric zone (Molina et al., 1974). However, part of this low dispersibility could be attributed to the formation of protein-phytic acid complexes as reported for navy beans at similar pH values (Powrie, 1961).

Results in Figure 1 further indicated that a large amount of the nitrogenous constituents can be extracted either by dilute HCl (about pH 2.0) or by dilute NaOH (at a pH higher than 8.0). It can be seen that the amount of nitrogen extracted was highest (81.84–85.28%) at the alkaline pH (8.0–12.0), and it was still higher than the percentage of nitrogen extracted at the neutral pH 6.8–7.0 (75.72–77.63%) or the acid pH values below 4.00 (58.89–79.39%). Moreover, the maximum nitrogen yield extracted was obtained at pH 11.05 (85.28%) as well as at pH 1.90 (79.39%).

The extraction profile of Figure 1 was generally in agreement with those reported for the same flour by Hang et al. (1970), Fan and Sosulski (1974), and Thompson (1977).

Effect of Temperature. Results for the effect of temperature on nitrogen extractability are given in Figure 2A.

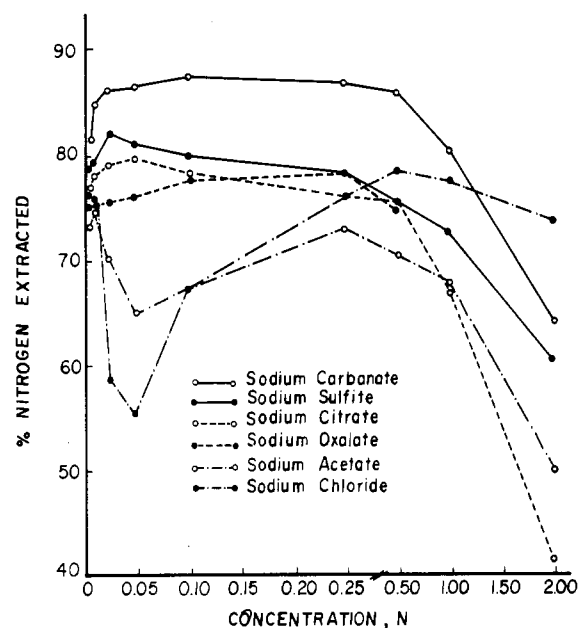


Figure 3. Effect of various salt concentrations on the nitrogen extractability of Iraqi whole mung bean flour.

These data indicated a small increase in the percentage of nitrogen extracted from 77.63% at 25 °C up to 79.85% at 60 °C with intermediate extractabilities in between. The results are comparable to those reported by Thompson (1977) for mung bean. He observed a slight increase in nitrogen extractability up to 60 °C with a decreasing yield at 80 °C due to partial coagulation of the proteins. Similar observations were also made by Gheyasuddin et al. (1970a) for sunflower seed proteins as percent nitrogen extracted dropped at 75 and 90 °C. Still, Molina et al. (1974) observed lesser increase in nitrogen extractability from Jack beans when temperature during the extraction was increased up to 80 °C.

Effect of Extraction Time. The influence of the duration of extraction (from 5 to 60 min) on nitrogen extractability from mung bean flour is given in Figure 2B. About 76.48% of the total nitrogen was extracted within 5 min from the beginning which indicated that the bulk of nitrogen dispersed rapidly. However, increasing the extraction time up to 25 min removed slightly more nitrogen and the percentage of nitrogen extracted increased from 77.25 to 79.54%. Longer extraction time (30–60 min) decreased extractable nitrogen to 77.40% (30 min) and 77.55% (60 min). In accordance with these data, Thompson (1977) reported that the time of extraction did not have appreciable influence on the nitrogen extractability from mung bean flour as all extractable nitrogen (88.2–88.8%) was in solution within 10–20 min. Other legume flours seem to behave similarly as reported by Gheyasuddin et al. (1970a) for sunflower seed and by Molina et al. (1974) for Jack beans.

Effect of Flour to Solvent Ratio. Nitrogen extractabilities with different flour to solvent ratios are given in Figure 2C. It is clear from these data that the ratio of 1:10 or less possessed little influence on nitrogen extractability, and the percent extractable nitrogen was found to remain more or less constant with nitrogen extractabilities within 77.15–77.63%. The ratio 1:5 showed small decrease in the extractable nitrogen to 75.33%. These findings corroborated those of Thompson (1977) for mung bean flour, and they are in good accord with conclusions made by Gheyasuddin et al. (1970a) for sunflower seed.

Salt Extraction. The effect of different salts on the nitrogen extractabilities from IWMB flour are presented

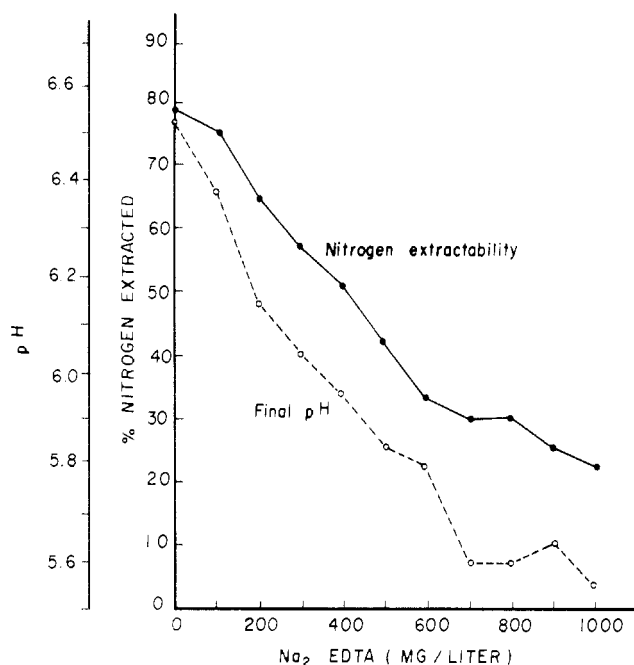


Figure 4. Effect of various concentrations of disodium ethylenediaminetetraacetate on the nitrogen extractability from Iraqi whole mung bean flour.

Table I. Final pH for Protein Extracts Obtained with Six Salts

concn, N	sodium chloride	sodium sulfite	sodium carbonate	sodium citrate	sodium acetate	sodium oxalate
0.000	6.52	6.52	6.52	6.52	6.52	6.52
0.005	6.60	6.95	8.50	6.75	6.75	6.60
0.010	6.55	7.40	9.40	6.75	6.75	6.70
0.025	6.55	7.50	10.20	6.80	6.80	6.75
0.050	6.50	7.55	10.40	7.05	6.80	6.75
0.100	6.40	7.70	10.75	7.05	6.90	6.75
0.250	6.30	7.70	11.05	7.10	7.05	7.00
0.500	6.20	7.75	11.20	7.10	7.25	6.95
1.000	6.10	7.75	11.30	7.15	7.50	
2.000	6.05	7.70	11.60	7.15	7.85	

in Figure 3 for sodium carbonate, sodium sulfite, sodium citrate, sodium oxalate, sodium chloride, and sodium acetate and in Figure 4 for Na₂EDTA. The final pH for every protein extract obtained except for Na₂EDTA is presented in Table I.

Except for sodium chloride, maximum nitrogen extractabilities attained by the salt extractants can be arranged on a decreasing order as follows: 87.58% with 0.100 N sodium carbonate, 82.22% with 0.025 N sodium sulfite, 79.93% with 0.050 N sodium citrate, 78.40% with 0.250 N sodium oxalate, 74.95% with 0.010 N sodium acetate, and 74.80% with 100 mg of Na₂EDTA. Interestingly, if arranged similarly, the pH of the protein extracts with these extractants will have the same decreasing order, e.g., 10.75, 7.50, 7.05, 7.00, and 6.75, respectively. Therefore, optimum salt concentrations producing the most alkaline final pH are also those which extract the most nitrogen. In accordance, Hang et al. (1970) concluded that alkaline salts were found to be fairly effective dispersing agents for mung bean proteins. However, the present work has indicated that alkaline salts concentrations higher than optimum values would increase pH but diminish extractability. Therefore, the correlation between pH of the salt extractant and N extractability cannot explain the complete extraction pattern.

For comparison of the extraction patterns of the seven salts of the IWMB flour, it can be divided into three major

types. The first type is characterized by an increase in their nitrogen extractabilities as the concentration of the salts was increased till they reached optimum concentrations. This type included sodium carbonate, sodium sulfite, and, to a lesser extent, sodium citrate and sodium oxalate. The second type is characterized by an appreciable inhibition to dispersibility at lower concentrations, followed by increase in N extractability, reaching a maximum at higher concentrations. Examples are NaCl and sodium acetate. The third type is characterized by a constantly dropping extraction profile as the salt concentration was increased. This type is exemplified by Na₂EDTA.

Salts of the First Type. Of this type, sodium carbonate and sodium sulfite had good similarity in their extraction patterns. When their concentrations exceeded optimum levels (e.g., 0.100 and 0.025 N, respectively) and reached 1.000 N, their N extractabilities dropped from 87.58 and 82.22% to 80.69 and 72.66%, respectively. Meanwhile, the final pH values of their protein extracts were increased from 10.75 and 7.50 to 11.30 and 7.75, respectively. The further increase in the salts concentrations to 2.000 N has caused further drops in their N extractabilities accompanied with additional increases in the pH of their protein extracts. However, sodium sulfite has also prevented the extensive darkening in the color of protein extracts occurring at pH 7 or higher, an observation which was made earlier by Gheyasuddin et al. (1970b). Moreover, Gheyasuddin et al. (1970a) concluded that sodium sulfite had extracted more nitrogen from sunflower seed meal at lower concentrations than at higher ones. Still, in accordance with the present work, Hang et al. (1970) concluded that the exact amount of nitrogen extractable by sodium carbonate from several beans was dependent upon concentration.

Sodium citrate and sodium oxalate have general similarity with the first-type salts with one exception. At their lower concentrations, they slightly inhibit nitrogen dispersibility. As their concentrations were increased beyond these levels, nitrogen extractabilities were increased to reach a maximum. A further increase in concentrations caused a drop in N extractabilities which was more drastic with citrate than with oxalate. The latter salt extraction profile was generally much less sensitive to changes in concentration. Still, the two salts were much less efficient protein extractants in comparison with sodium carbonate and sodium sulfite. Moreover, they have affected small increases in pH over wide ranges of concentrations. Comparable trends for the extraction pattern of sodium citrate were given by Hang et al. (1970). However, the available literature on sodium oxalate is very scarce.

Salts of the Second Type. Sodium chloride and sodium acetate retarded nitrogen solubility at lower concentrations. The increase in NaCl concentration from 0 to 0.050 N caused N extractability to drop from 79.00 to 55.45% compared with a lesser drop to 73.43% in the case of sodium acetate at 0.005 N. The two salts were poor protein extractants with sodium chloride being slightly better. Besides, NaCl has changed the final protein extract pH only slightly while sodium acetate caused a small increase from the initial 6.52 to 7.85 at 2 N concentration. Maximum nitrogen extractabilities were 78.78% at 0.500 N NaCl and 74.95% at 0.010 N sodium acetate compared with 79.00% with water alone. Comparable data for NaCl was given by Smith et al. (1959). However, Hang et al. (1970) reported that NaCl at 0.75 N extracted slightly more nitrogen than water alone. Still, Gheyasuddin et al. (1970a) reported a different extraction pattern for sodium acetate with sunflower seed meal. It was characterized by an

increase in the percent nitrogen extracted as the concentration of sodium acetate was increased.

Salts of the Third Type. When the concentration of Na_2EDTA was increased from 0 to 1000 mg/L (Figure 4), nitrogen extractability dropped from 79.00 to 22.18%. The pH of the protein extracts (Figure 4) were also constantly dropping with the increase in salt concentration from an initial value of 6.52 to a minimum value of 5.55. This pattern was unique in comparison with those of other salts as it had only one dropping trend. It seems that the acidic effect of the salt had the predominant effect in inhibiting extractability by making the medium more acidic and thus closer to the pH range of minimum protein solubility. Therefore, other salts with some acidic effect would probably fit to the same type. However, the chelation of divalent cations with Na_2EDTA and its presumed effect on protein dispersibility was not evident under the experimental conditions used. Unfortunately, the experiments were not repeated at pH 7 or higher. Besides, the available literature on the effect of Na_2EDTA on protein extractability from legume flours is scarce.

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Isolation and Identification of Volatile Compounds in Cooked Meat: Sukiyaki

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The sukuyaki volatiles obtained from beef, heated with vegetables and seasonings (sugar and soy sauce) under simulated cooking conditions, were isolated and identified by gas-liquid chromatography/mass spectrometry. Forty-four compounds were positively identified. This included furans, ketones, aldehydes, esters, acids, alcohols, pyrazines, hydrocarbons, and pyrroles. Dimethylnitrosamine was identified at a level of 0.02 ppb in the dichloromethane extract of the above sukuyaki broth by using a thermal energy analyzer (TEA) combined with a gas chromatograph.

There are many reports on the isolation and identification of cooked meat constituents (Persson and von Sydow, 1973; Mussinan and Walradt, 1974; Buttery et al., 1977). All studies have been based upon the analysis of a single meat sample. When meats are cooked, however, they are often heated with many other ingredients such as vegetables, cheese, seasonings, etc. The many volatile compounds in cooked foods may form from the reactions between the constituents of the various ingredients used.

Sukiyaki is one of the typical Japanese one-pot dishes. Thin-sliced beef is fried together with vegetables, usually (nitrate-rich) chinese cabbage or spinach (in a frying pan at the table). Fairly large amounts of sucrose and soy sauce are used as seasoning.

Soy sauce contains large amounts of various amino acids (Markley, 1951), and sucrose produces many carbonyl compounds by heat treatment; together (Hodge, 1967) they can form browning reaction products during the frying. The unique flavor of sukuyaki may come from the volatile browning reaction products or from secondary reactions

with other food components.

Recently, carcinogenic *N*-nitrosamines have been isolated and identified in a wide range of foodstuffs: canned meat (Sen et al., 1976), smoked beef (Stephany et al., 1976), sausages (Kann et al., 1976), and cooked bacon (Patterson and Mottram, 1974). Using sodium nitrite for curing meats is a major source for *N*-nitrosamine formation (Freimuth and Glaeser, 1970; Moehler and Mayrhofer, 1969; Sen, 1972).

N-Nitrosamines form when secondary amines react with nitrite. Since meats contain various secondary amines, for example, dimethylamine, diethylamine, and *n*-diisopropylamine (Golovnya et al., 1979), it seems likely that during the cooking process these amines can react with the nitrite in the cured meat. Small amounts of nitrite can also be generated from nitrate-rich vegetables when they are heated with other food ingredients (Hayano, 1976).

In this study, the volatile browning reaction products produced in sukuyaki, which was prepared under simulated home cooking conditions, were isolated and identified. The detection of *N*-nitrosamines in sukuyaki flavors was conducted using a Thermal Energy Analyzer.

EXPERIMENTAL SECTION

Preparation of Sukuyaki Volatiles. Beef (400 g), *Cryptotaenia japonica* Hassk (mitsuba, 60 g), *Brassica*

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