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IDENTIFICATION OF SOY PROTEIN IN MEAT BY PYROLYSIS–HIGH-RESOLUTION GAS CHROMATOGRAPHY

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SUMMARY

Pyrolysis–gas chromatography was applied to the characterization of ground beef and soy protein isolate and its mixtures at the 50, 30 and 10% levels. A cryogenic trap pyrolysis mode was employed and the volatile pyrolysates were analysed by a high-resolution capillary gas chromatograph. Two phenolic derivatives, *o*-methoxy- and dimethoxyphenol, as well as 2,3-dithiabutane are uniquely observed at a large concentration in the soy pyrolysate. Quantification of these characteristic compounds allow the estimation of the level of soy inclusion in the mixture. This approach effectively denotes soy addition up to the 10% level in comminuted beef.

INTRODUCTION

The availability of low-cost non-meat protein sources make such components an attractive meat substitute. Soybean protein is one of the vegetable proteins that is a frequent addition in meat products. Ground beef may be formulated with up to 25% of hydrated soy protein as an extender and/or binder. Legal authorization also extends to the school lunch program where up to 30% hydrated vegetable protein (typically soy protein) may be added to meat. Consequently, there is a need for the accurate characterization and quantitation of soy protein in meat products.

Many methods have been proposed for the identification of soy protein in meat mixtures[1,2]. Olsman[3] lists these in five separate categories based on two broad groups: (1) techniques involving the presence of substances accompanying the protein, and, (2) those that examine the protein itself. Chemical methods include determination of the amino acid canavanine, (uniquely associated with soy), manganese, magnesium and phytic acid. Generally, a low degree of specificity is associated with this approach. Microscopic techniques, based on the characterization of unique cell types, are effective only when the whole soybean is involved. In such cases, differentiation is based on the presence of unique morphological cell-types such as the pallisade and hour-glass cells or by differential staining techniques involving the polysaccharide cell wall of the bean. Soy protein isolate, on the other hand, has not been demonstrated to be microscopically detectable in meat and soy protein mixtures.

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The poor reliability of the quantitation process in electrophoretic methods makes this technique unfeasable despite the high degree of specificity involved. Immunochemical methods offer high specificity and sensitivity and consequently show a great potential for such use. Methods based on the amino acid composition or sequence also show a great deal of promise but have yet to be proven effective in the qualitative and quantitative determination of soy and meat protein mixtures.

In the last two decades, analytical pyrolysis has been applied to the identification of a number of biological materials. Early studies, primarily those by Reiner[4] on the characterization of microorganisms have shown distinctions between microbial species and even microbial strains. More recently, differences have been established for various enzymes and isoenzymes[5], fungi[6] and even between human organ tissues[7,8]. These and other investigations have demonstrated the highly sensitive and selective nature of both pyrolysis–gas chromatography (Py–GC) and pyrolysis–mass spectrometry (Py–MS). Collaborative studies have also established this technique as a highly reliable one based on the excellent reproducibility of data generated[9,10].

This paper describes the use of Py–GC in the characterization and quantitation of soy protein isolate individually and in mixtures with beef muscle protein. The approach taken involves a novel model of pyrolysis, termed cryogenic trap pyrolysis.

EXPERIMENTAL

Sample preparation

Fresh beef muscle tissue (100 g) was homogenized in a blender and extracted with 500 ml of acetone. The process was repeated three times. This was followed by repeated (usually 1–2) hexane extractions until a fat free residue was obtained. The mixture was centrifuged at 8000 *g* for 1 h and dried in a vacuum oven at 45°C for 5 h. The dried product was then ground and stored in a moisture-free environment.

Soy protein isolate was purchased from Central Chemurgy (Chicago, IL, U.S.A.). Mixtures of soy protein isolate in beef muscle were prepared at 50, 30 and 10% soy levels.

Pyrolysis

The sample was conducted in a 300-ml three-necked flask that was fitted with a thermometer and a source of inert nitrogen gas. The headspace volatiles were swept into a series of glass traps consisting of a 500-ml vessel (air cooled), a U-tube water trap and a series of four coiled traps (all cooled with a dry ice–acetone slurry). A flow meter was placed at the end of the final trap. Nitrogen flow-rate was set at 30 ml/min while the heating temperature was maintained at 200 ± 2°C.

GC analysis

Samples were extracted from the traps with methylene chloride and dried over anhydrous sodium sulfate. They were then concentrated to a final volume of 1–2 ml (using a combination of rotary evaporation and flushing with nitrogen gas) and analysed in a Varian Model 3400 capillary gas chromatograph fitted with a 30 m × 0.25 mm I.D. fused-silica Carbowax 20M. Profiles were obtained using a SP4270 recording integrator. Conditions employed in the GC runs were as follows: injection size, 1 μl; initial temperature, 60°C for 2 min to a final temperature of 220°C at

4°C/min. Flow-rate was maintained at 1 ml/min with helium as the carrier gas. A flame ionization detector set at an attenuation of 4 and a detector range of 10^{-11} A/mV was used. The plotter speed was set at 2.0 cm/min.

Mass spectrometry

A VG 7070 EQ mass spectrometer with the INCOS data system was employed in this study. This was fitted with a Hewlett-Packard 5890 gas chromatograph for the GC–MS analysis. The analysis was conducted at a filament voltage set at 70 eV, the acceleration voltage at 6 kV and a trap current of 100 μA.

RESULTS AND DISCUSSION

Beef pyrolysate

Pyrograms obtained from the cryogenic trap pyrolysis of pure beef muscle protein and soybean protein are presented in Fig. 1A and B. The beef protein pyrogram in this study exhibits a larger total number of components (206) than that of soybean (176). Data obtained from the integrator denotes a total of 77 beef muscle pyrolysate components not observed in the soy profile. While many of these are the less intense constituents, beef muscle protein can clearly be characterized also by the appearance of a number of moderately to extremely intense peaks. Twenty five components in this category can be discerned in Fig. 1A. The largest and most significant peaks unique to beef muscle are represented by the letter (S). Their appearance is also frequently observed in each of the mixed protein profiles. One observation here is that the majority of these beef muscle associated compounds elute in the early half of the pyrogram, classifying them as low boiling, non-polar to moderately polar constituents. In the beef–soy mixtures, these peaks show an increase in intensity as the level of beef in the mixture is increased. This observation confirms the origin of these components as arising solely from the beef protein pyrolysate. In addition to the presence of unique constituents, beef muscle pyrolysate also consists of a number of components appearing at much greater intensities that the corresponding peak in the soybean pyrolysate. A total of five quantitatively unique peaks are identified in this profile and are represented by the letter (L) in Fig. 1A. They serve to distinguish the animal from the vegetable protein.

The chemical nature of some of these compounds, particularly those not observed in the soy pyrolysate has been determined by GC–MS. The trisulfide, 2,3,4-trithiapentane appearing at a large concentration, is one characteristic feature of this muscle pyrolysate. Its formation via the degradation of cysteine and interaction with hydrogen sulfide is not observed at a significant level in the soy profile.

Other compounds identified include the 4-phenyl pyridine, decyl acetate, 2-butylthiophene, 2-*n*-pentylthiophene and methyl stearate all of which do not occur in the soy pyrolysate. In general, the pyrolysate composition of both muscle and soy consists of a number of carbonyls (particularly ketones) and the sulfur- and nitrogen-containing heterocyclics under the conditions employed in this experimental study.

Soybean pyrolysate

Soybean protein pyrolysate shows a total of 73 peaks that are not observed in

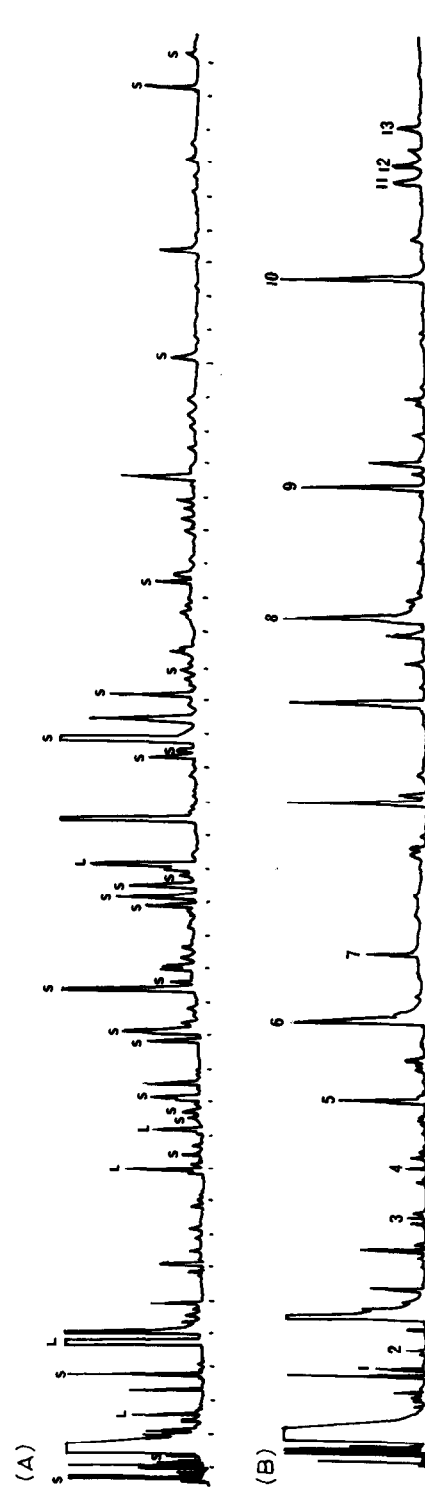

Fig. 1. Beef muscle (A) and soy protein (B) pyrograms. S = Not observed in soy pyrolysate; L = much larger in beef pyrolysate; 1–13 = components unique to soy.

the beef pyrolysate. As in the case of the beef protein, the majority of these are of low intensity. However, thirteen peaks of moderate to large intensities can clearly be recognized in this profile. These are represented by peaks 1–13 in Fig. 1B. An interesting aspect of this profile is that, unlike the situation in beef protein, the elution times of many of these components and particularly the most intense ones, such as peaks 6, 8, 9 and 10 and are observed in the latter half of the profile and may probably be attributed to strongly polar, high-boiling-point compounds. In addition, a number of moderately intense components are also characteristic of the presence of soy protein and are expectedly observed in the mixtures, particularly at the larger soy levels.

Two phenol-based compounds have been identified, the *o*-methoxy- and dimethoxyphenol. Phenolic compounds are characteristic of plant tissues such as soybean and cottonseed. In the soy pyrolysate these compounds are observed at a relatively large concentration but are totally absent in the beef muscle pyrolysate. Another compound seen at a large concentration in soy pyrolysates is 2,3-dithiabutane. The origin of this compound can be expected from the amino acid cystine.

The absence of this disulfide in the beef profile is surprising since the level of cysteine in muscle is significantly greater than in soy protein (however, the position of the amino acid in the protein molecule is also a significant factor). Muscle pyrolysates, however do contain large quantities of the trisulfide, trithiapentane that is observed only at minor levels in soy. It is expected that the formation of the trisulfide is encouraged by the abundant presence of hydrogen sulfide generated from the thermal degradation of the sulfur-containing amino acids, particularly cysteine which is the most thermally labile of the amino acids;

$$R\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}R + H_2O \rightarrow R\text{-}CH_2\text{-}SH + R\text{-}CH_2\text{-}SOH$$
$$R\text{-}CH_2\text{-}SOH \rightarrow R\text{-}CHO + H_2S$$

Beef-soy pyrolysates

In order to establish the applicability of Py–GC for the purpose of quantifying soy in beef protein, three levels of mixture were studied (50, 30 and 10%). The 50% beef–soy mixture denotes a total of 53 peaks not seen in the pure beef program. All the major components unique for soy protein, with the exception of peak number five are learly visualized in this profile (peaks 1–13 in Fig. 2A). One interesting aspect of this and the other mixtures involves the appearance of unique peaks that were not earlier observed in either of the individual pure protein pyrolysates. In the 50% mixture, this group is represented by a total of 25 compounds. Of these, peaks a, b and c (Fig. 2A) are the strongly intense ones and can clearly be discerned from the complex chromatographic profile of this mixture. Their occurrence may be attributed to the pyrolysates formed solely from the interaction of the primary reaction products of the two protein samples. Neither the beef nor the soy protein individually generate any of these compounds. fifty total soy-associated compounds characterizes the 30% soy–beef mixture of which most of the peaks unique to soy are again observed (Fig. 2B). The intensity of most of these peaks are weak with the exception of peaks 6–10. Of an overall 20 peaks unique to the mixture, peaks a and c remain as the strongly intense components not observed in either of pure protein states. Overall, at this 30% level, a slightly reduced total number of soy-related components is observed. Finally, at the 10% mixture level, the overall profile approaches that of the pure beef pyro-

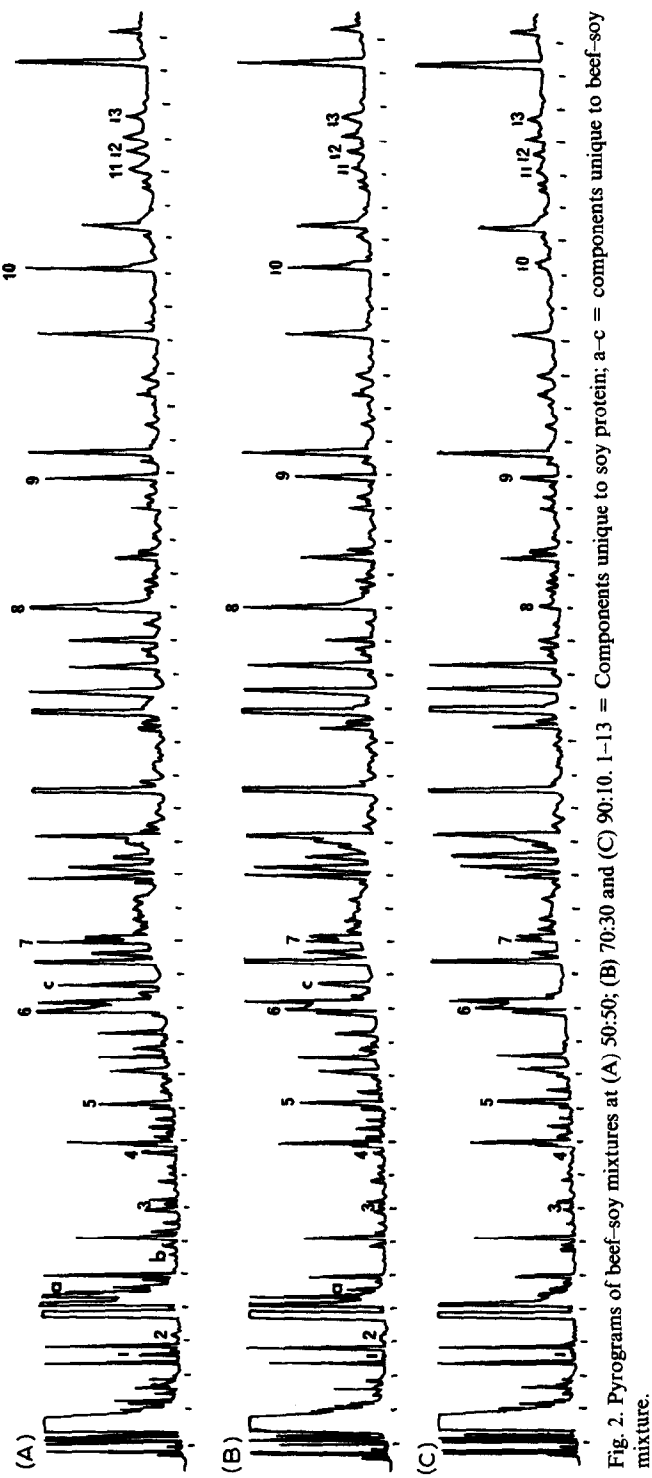

Fig. 2. Pyrograms of beef–soy mixtures at (A) 50:50; (B) 70:30 and (C) 90:10. 1–13 = Components unique to soy protein; a–c = components unique to beef–soy mixture.

gram. Yet this mixture again clearly can be differentiated from the beef profile. Overall, 45 non-beef protein peaks are observed, while a third of these are uniquely associated with soy–beef mixtures. Peaks are generally of reduced intensities with the exception of the moderately intense peaks 6, 7, 8 and 9. Peaks unique to the mixture are now difficult to visualize at the 10% level (Fig. 2C). Replicate analyses of these tissues have generated excellent reproducible data in each case, with variations generally below the 5% level.

The five most intense peaks in soy protein pyrograms 6, 7, 8, 9 and 10 are also clearly observed at each level of mixture tested. More importantly, when the area of these peaks (normalized) are plotted against the soy–beef mixture compositions, a linear relationship is observed between peak intensity and the amount of soy protein present in the mixture. Regression values obtained in each case demonstrates a high correlation factor as observed in Fig. 3. This allows for the estimation of the level of component present in the mixture based on the intensity of these appearing peaks in the chromatographic profile.

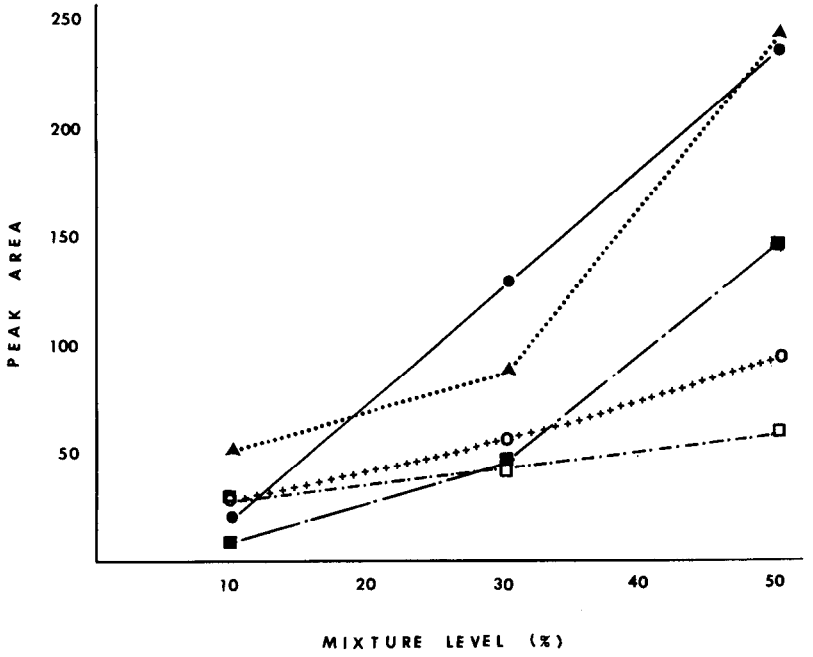

Fig. 3. Relationship of protein mixture level to peak area for peaks 6 to 10. Key to symbols: ▲ = peak 6, r = 0.9394895; ○ = peak 7, r = 0.9999681; ● = peak 8, r = 0.9961957; □ = peak 9, r = 0.9986254; ■ = peak 10, r = 0.9721792.

Chromatographic profiles generated from previous studies on pyrolysis products are frequently complex and involve the observation of subtle differences. Such differences are basically quantitative in nature and are extremely difficult to visualize for the untrained person. Special computer-aided co-applications are a necessity for discrimination between two or more of these pyrograms[9]. Recent applications have even abondoned the chromatographic approach in pyrolysis altogether in favor

the more sensitive (but more specialized and costly) approach of pyrolysis–mass spectrometry[8,11].

The difficulty of employing high-resolution gas chromatogramphic (HRGC) analysis is primarily because the commercially available pyrolyzers employ high carrier gas flow-rates in channelling the products into the gas chromatograph. This necessitates the use of the split injection mode in HRGC units interfaced on-line with the pyrolyzers. A resulting loss of the overall number of components is now experienced since many compounds in the mixture are present at too low levels to be detected. Clearly there is a demand for some modification to existing systems (if possible) or a total redesign or perhaps the introduction of novel thermal degradation modes. This study employs HRGC in a mode suited for its application. With the increased resolution obrtained, the focus can be purely on the qualitative differences that are rapidly determined (through their characteristic retention times) thereby avoiding the need for computer applications. Consequently, the effectiveness of this approach as a quantitative test for evaluating the presence of soy in meat is achieved.

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