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Received for review August 28, 1978. Accepted January 16, 1979.

^{13}C–^{12}C Analysis of Vegetable Oils, Starches, Proteins, and Soy–Meat Mixtures

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The ^{13}C–^{12}C ratios for a number of vegetable oils, starches, and proteins have been determined. As expected, the values for animal proteins reflect the animals' diet. The possible application of using ^{13}C–^{12}C analysis in differentiating corn-fed animal protein (C_4 plant) from soy protein (C_3 plant) in soy–meat mixtures is discussed.

Measurements of the relative abundance of the naturally occurring stable carbon isotopes ^{12}C and ^{13}C have proven to be an important tool for evaluating the importance of specific chemical and physical processes in biochemical and geochemical cycles. Processes which discriminate against the heavier ^{13}C and favor use of the lighter ^{12}C cause isotopic fractionation to occur. With the development of sensitive mass spectrometric techniques, variations in the ^{13}C–^{12}C ratios of one part in ten thousand can be detected (Craig, 1953). Determination of ^{13}C–^{12}C ratios in organic matter may be carried out by combustion of relatively small amounts of material (5–10 mg) to CO_2. The ease with which CO_2 can be collected and purified has allowed stable carbon isotope measurement surveys to be undertaken in the biosphere and geosphere (Friedman and Irsa, 1967; Lerman and Troughton, 1976).

Surveys on the natural variations of ^{13}C–^{12}C ratios in the plant kingdom (Craig, 1953; Lerman and Troughton, 1976; Lerman, 1972; Minson et al., 1975; Bender, 1968; Smith and Brown, 1973; Tregunna et al., 1970; Lowdon, 1969; Garnier-Dardart et al., 1976; Smith and Epstein, 1971) have shown that the bimodal distribution of stable carbon isotope ratios (^{13}C–^{12}C) found to occur naturally in higher plants correlates closely with the two photosynthetic routes used by these plants in the fixation of atmospheric carbon dioxide. These biochemical routes, known as the C_3 (Calvin-Benson) and C_4 (Hatch-Slack) photosynthetic pathways, involve the initial fixation of CO_2 into respectively a three-carbon and four-carbon dicarboxylic acid product during the enzymatic carboxylation reaction. With average differences of approximately 14 parts per thousand (ppt) observed in the corresponding $\delta^{13}C$ values, the stable carbon isotope ratio is a direct indication of the carbon pathway used by the higher plant. Thus plants and plant products which are C_3 (such as wheat, oats, barley, potatoes, and soybeans) are readily distinguished from C_4 plants and plant products (such as sugar cane, corn, and sorghum) by their ^{13}C–^{12}C ratios.

The occurrence of natural ^{13}C labeling in plants and plant products has found application in many research investigations ranging from glucose metabolism studies in man (Lacroix et al., 1973) to substantiating incorporation of maize cultivation into an early North American culture (Vogel and Van Der Merwe, 1977).

Use of stable carbon isotope techniques may have direct applications in the food and drug industry (Lerman and Troughton, 1976; Haines, 1976). For example, the adulteration of carbohydrate products such as natural syrups and honeys by the undeclared addition of sugar cane or corn-derived products may be monitored using ^{13}C–^{12}C measurement (Doner and White, 1977).

Food chain investigations have shown that natural ^{13}C–^{12}C ratios of animal tissues (Minson et al., 1975; Parker, 1964; De Niro and Epstein, 1978) [as well as those of human tissues (Gaffney et al., 1978)] tend to follow

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0021-8561/79/1427-0475$01.00/0

closely the ratios of the animals' food sources. It follows that animal protein products, such as beef and pork from livestock which are fed principally on C_4 plant feeds such as corn, may be differentiated from those fed soy protein and other C_3 plant proteins.

This paper presents ^{13}C–^{12}C ratios determined for a number of protein, starch, and oil samples that should be of use in determining the feasibility of using stable carbon isotopic measurements in commercial food mixture analysis. The possible use of ^{13}C–^{12}C ratios in determining amounts of soy protein in beef, pork, and other protein feeds will be discussed.

EXPERIMENTAL SECTION

^{13}C–^{12}C Isotope Ratio Determinations. The experimental procedure has been previously described, but it will be briefly reviewed here (Gaffney et al., 1978; Slatkin et al., 1978).

Samples (typically 5–10 mg) were placed in small Pyrex boats (10 × 20 mm) and weighed to the nearest 0.1 mg. After positioning the boats in a low-temperature asher (LFE Corp., LTA Model 302), the samples were combusted in an oxygen plasma. The low-temperature asher (LTA) was used in place of the more conventional combustion furnace to enable a determination of the trace elements in the ash to be carried out if this should prove to be desirable. For CO_2 collection, the LTA system was modified by replacing all silicone rubber tubing with stainless steel, copper, and Pyrex tubing. The modified LTA system was operated without sample for several hours to remove traces of grease and other inorganic impurities until carbon-free blanks were obtained. The LTA was operated using 200-W microwave power with a gas pressure of 1–3 torr. Water-saturated oxygen (~20% of LTA gas flow) was bled into the main stream of oxygen to optimize combustion-discharge conditions. The LTA effluent gas was run through a vertical copper oxide column at 300 °C to promote conversion of CO to CO_2 and then exhausted through a series of cold traps at −77, −77, and −196 °C.

The gas collected in the final trap was passed through a gas chromatograph using a 1 ft × $^1/_4$ in. molecular sieve 13X, 80–100 mesh column, at 138 °C in series with a 15 ft × $^1/_4$ in. 20% DC-710 silicone oil (Dow Corning) on firebrick (80–100 mesh) column held at 72 °C to separate and purify the CO_2. This is necessary to separate the CO_2 from oxides of nitrogen and sulfur which are principal contaminants. Typical helium carrier flow rates through the columns and thermal conductivity detector were about 30 mL/min. After chromatography, CO_2 was trapped at −196 °C, freeze-thawed to remove traces of helium, and then measured on a calibrated vacuum line as an ideal gas at STP. The measured CO_2 was trapped in a collection flask and transferred manually to an Atlas Werke M86 mass spectrometer.

The ^{13}C–^{12}C isotope ratios were determined by mass spectral comparison of the $^{13}C^{16}O_2$ and $^{12}C^{16}O_2$ ion beams (Friedman and Irsa, 1967). The data are presented as $\delta^{13}C$ values, where

$$\delta^{13}C(\text{ppt}) = \frac{(^{13}C\text{–}^{12}C)_s - (^{13}C\text{–}^{12}C)_{PDB}}{(^{13}C\text{–}^{12}C)_{PDB}} \times 1000$$

$(^{13}C\text{–}^{12}C)_s$ and $(^{13}C\text{–}^{12}C)_{PDB}$ being the isotope ratios of sample and reference standard (PDB refers to CO_2 produced from standard calcium carbonate Peedee formation belemnite, Upper Cretaceous, S.C.), respectively. Ratios were corrected for the $^{12}C^{16}O^{17}O$ contribution to the 45 m/e signal used to measure $^{13}C^{16}O_2$ (Craig, 1957). Periodically, samples of graphite were combusted and ^{13}C–^{12}C ratios were determined as a check on the experimental system reproducibility. By running reference CO_2 through the gas chromatographic system, it was found that no isotopic fractionation occurred during CO_2 purification. Using this experimental system human tissue samples for an individual specimen were found to be reproducible to a standard deviation of ±0.55 (Slatkin et al., 1978).

The types of samples and the methods by which they were processed prior to ashing are described as follows.

Wheat Protein Samples. Protein from a hard red winter wheat (Ponca variety) flour was fractionated into globulin, gliadin, and glutenin by previously described alcohol and salt extraction procedures (Jones et al., 1959).

Corn Protein Samples. Protein from hexane-defatted corn (DeKalb variety XL66) flour was fractionated into glutelin, globulin, and zein by previously described alcohol and salt extraction procedures (Boundy et al., 1967).

Soy Protein Samples. Kanrich variety (1971) certified soybeans were cracked, dehulled, flaked, and extracted in a Soxhlet extractor with hexane to yield defatted soybean meal. Soy protein concentrate (70% protein) was obtained from Central Soya Company, Chicago, IL. The soy protein isolate (90% protein) was a commercial sample from Ralston Purina Company, St. Louis, MO.

Meat Samples. Center-cut pork chops from a local supermarket were deboned and freeze-dried. Large pieces were shredded by hand and then extracted in a Soxhlet with hexane to give defatted pork, which was ground in a Wiley Mill to 60 mesh.

To obtain a defatted beef sample, round steak was treated the same as the pork sample.

Corn Starch Samples. Immature starch granules were extracted 12 days after pollination from Golden Cross Bantam sweet corn by steeping in alkaline-distilled water. Extracted starch granules were defatted by Soxhlet extraction for 48 h with 85% aqueous methanol (Wolf et al., 1948). Immature starch granules were also extracted 18 days after pollination.

Amylose corn starch was isolated from an Iowa hybrid 939 variety by steeping 23 h in distilled water at 55 °C. The starch paste was defatted with 85% aqueous methanol and then treated in sulfuric acid at 50 °C to reduce viscosity of paste. After autoclaving, the paste was fractionated and recrystallized two times from 1-butanol saturated water (Schoch, 1941, 1942).

Thatcher wheat was steeped in distilled water for 24 h at 34 °C, ground to flour, screened, and centrifuged to isolate starch (MacMasters and Hilbert, 1944).

Waxy sorghum starch was isolated from sorghum flour (*Sorghum vulgare*). The flour was ground without steeping in a Buhler Mill, sieved, and centrifuged to isolate the starch.

Fat and Oil Samples. Beef tallow–freeze-dried round steak was ground to 60 mesh and extracted with hexane. Hexane was removed under vacuum with a rotaevaporator. Coconut, soybean, sunflower, corn, palm, and peanut oils were obtained directly from commercial manufacturers. Fatty acid composition of the oils was determined by gas chromatograph and used to confirm the authenticity of the samples (Spencer et al., 1976).

RESULTS AND DISCUSSION

Stable carbon isotope ratios determined for a number of protein, starch, and fat and oil samples are given in Table I. The $\delta^{13}C$ values measured for beef (−13.1 ± 1.6) and pork (−12.5 ± 0.4) proteins are consistent with the heavy use of corn and sorghum as livestock feeds in the United States. These data, when compared with $\delta^{13}C_{PDB}$ values determined for beef from southern Scotland (−28.8 ± 3.1) (Harkness and Walton, 1972), where C_3 grasses are

Table I. Relative Carbon-13 Content of Various Protein, Starch, and Oil Samples

	no. of analyses[a]	$\delta^{13}C$[b]
Proteins		
defatted beef	9	−13.1 ± 1.6
defatted soybean meal	8	−22.4 ± 1.1
defatted pork	6	−12.5 ± 0.4
soy protein concentrate	5	−21.8 ± 1.6
soy protein isolate	3	−23.6 ± 0.6
wheat globulin (Ponca), salt-soluble fraction	1	−22.0
wheat gliadin (Ponca), alcohol-soluble fraction	1	−23.6
wheat glutenin (Ponca), salt- and alcohol-insoluble fraction	1	−23.7
corn glutelin (DeKalb XL66), salt- and alcohol-insoluble fraction	1	−5.2
corn globulin (DeKalb XL66), salt-soluble fraction	1	−8.6
corn zein (DeKalb XL66), alcohol-soluble fraction	1	−12.6
wheat gluten (Ponca)	1	−23.0
Fats and Oils		
beef tallow	3	−16.6 ± 0.1
coconut oil	3	−25.5 ± 0.5
soybean oil	3	−28.1 ± 0.4
sunflower oil	3	−27.3 ± 1.0
corn oil	3	−12.4 ± 0.6
palm oil	3	−27.0 ± 0.4
peanut oil	3	−27.6 ± 0.3
Starch Samples		
corn (defatted starch 12 days after pollination)	1	−5.4
corn (defatted starch, 18 days after pollination)	1	−7.5
corn (amylase starch fraction)	1	−3.2
wheat starch (Thatcher)	1	−21.0
waxy sorghum starch (*Sorghum vulgare*)	1	−6.1

[a] Represents repeated analysis of portions of the same sample. [b] Error limits are standard deviations.

principal feeds, support previous interpretations that the $\delta^{13}C$ values measured for animals are closely related to their major food sources (Minson et al., 1975; Parker, 1964; Gaffney et al., 1978; De Niro and Epstein, 1978). This reinforces the assumption that biochemical fractionation of carbon in the food chain is small when compared to the initial photosynthetic fractionation by the higher plants. The values observed for these United States animal protein sources are also consistent with previous findings, that $\delta^{13}C$ values for human tissues obtained from Long Island autopsy cases differ significantly from $\delta^{13}C$ values measured for comparable European tissues (Gaffney et al., 1978).

Note that the standard deviations for the oil samples are significantly less than those obtained for the powdered protein samples. This most likely reflects the problems in obtaining a representative sample from a powder where inhomogeneities are likely.

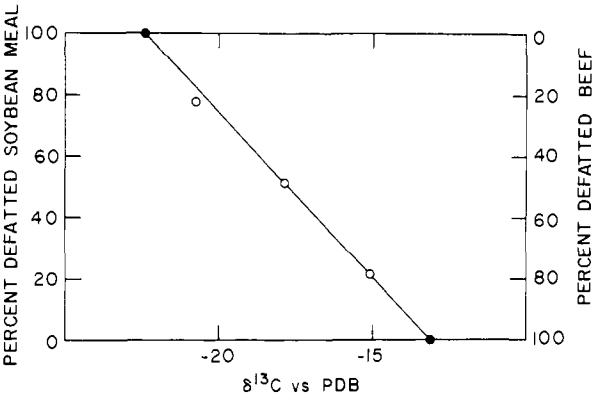

Figure 1. Calibration data for relative amounts of defatted soybean meal protein and defatted beef (● = values for pure materials taken from Table I, ○ = 20:80, 50:50, and 80:20 mixtures, one determination).

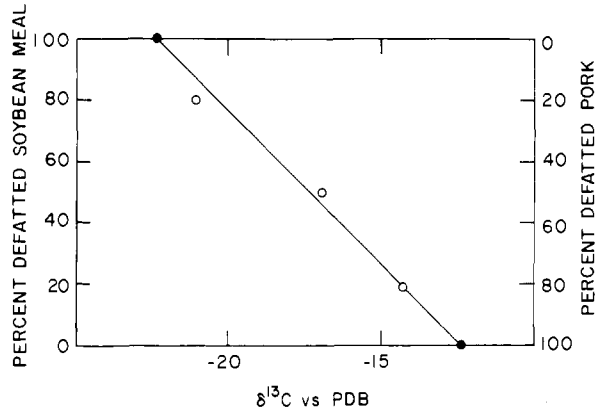

Figure 2. Calibration data for relative amounts of defatted soybean meal protein and defatted pork (● = values for pure materials taken from Table I, ○ = 20:80, 50:50, and 80:20 mixtures, one determination).

To determine if a mixture of two powdered proteins could be analyzed for the relative amount of the materials, using stable carbon isotope techniques, soy–meat samples were prepared at NRRC, USDA.

The soy–meat samples consisted of mixtures of defatted soybean meal and defatted beef or defatted pork. Defatted soy–meat mixture, standard calibration curves were obtained by analyzing known 20:80, 50:50, and 80:20 mixtures of the defatted proteins. Because of sampling problems, the $\delta^{13}C$ value was determined by combusting the entire sample. These data are presented in Figures 1 and 2 for mixtures of defatted soybean meal–defatted beef and defatted soybean meal –defatted pork, respectively.

Based on these experiments, results of the analyses of the three unknowns are presented in Table II. These results indicate that this technique may be feasible for such analyses. Note that, within reasonable error limits, the actual weight percent of soybean meal and the value determined using ^{13}C–^{12}C isotopic analysis are in good agreement. The accuracy of such determinations may

Table II. Calibration Data for Blend Analysis

	no. of analyses	$\delta^{13}C$[a]	wt % defatted soybean meal	measured %[b] defatted soybean meal
blend of defatted beef and defatted soybean meal	5	−16.6 ± 1.3	40.5	38 ± 11
blend of defatted beef and defatted soybean meal	4	−15.2 ± 0.6	31.8	23 ± 7
blend of defatted pork and defatted soybean meal	6	−15.2 ± 2.1	24.5	27 ± 4

[a] Error limits are standard deviations. [b] Using calibration data from Figures 1 and 2. Error limits are based on data from Table I for pure samples.

certainly be affected by the problems in sampling a mixture of powders. Increasing the sampling size or homogenizing the sample by liquefaction or some other process may lower the error limits in these analyses. One should also be cautious of isotopic fractionation that could occur from the extraction processes used to remove lipid fractions from these samples. Adsorbed solvent from such extractions could also lead to errors in the ^{13}C–^{12}C determinations.

With this in mind, these data allow for the estimation of the accuracy of measurements using ^{13}C–^{12}C techniques on inhomogeneous mixtures. The use of this technique to measure relative amounts of soy protein in meat products appears to be possible with errors of ~10% expected. The ^{13}C–^{12}C ratios for individual pork and beef samples would be expected to show some variance depending on the proportion of soybean protein, corn, and grasses in their diet, and therefore it will be necessary for a substantial data base to accumulate in the literature before routine use can be made of ^{13}C–^{12}C ratio variations. It is evident from the foregoing discussion that this method of differentiating between soybean protein and beef or pork protein would not be useful when the livestock are fed primarily on C_3 feeds.

ACKNOWLEDGMENT

The technical assistance of Doris Franck is gratefully acknowledged. Starch samples were supplied by J. E. Hodge. Fat and oil samples were supplied by G. R. List. Wheat and corn protein samples were supplied by J. S. Wall. Soy protein, meat protein, and soy–meat blend were supplied by A. C. Eldridge.

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Received for review August 16, 1978. Accepted December 11, 1978. This research was carried out at Brookhaven National Laboratory under contract with the U.S. Department of Energy and supported by its Division of Biomedical and Environmental Research.

Kinetics of the Production of Biologically Active Maillard Browned Products in Apricot and Glucose–L-Tryptophan

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Some properties of those Maillard browning products responsible for these adverse effects were studied. The kinetics of the formation of these brown products was also studied using a natural food system (apricot) and a model system (glucose–tryptophan). The water-soluble products responsible for the deterioration of the normal nutritional state were formed in the early stages of browning. Butanol-soluble products attributable to adverse physiological effects were formed in a later stage. Kinetically, a significant proportion of the parent compounds were degraded, and a maximum yield of Amadori compounds was attained even before an appreciable amount of brown color developed. The rate of formation of browning products showed a linear relationship with reaction time and temperature until the parent compounds were no longer available. After depletion of parent compounds, polymerizations between the remaining products and a partial degradation of Amadori compounds occurred. The products became less soluble in polar solvents as further polymerization proceeded.

The nonenzymatic Maillard browning reaction is known to cause the deterioration of the quality of food products during processing and storage. Along with the loss of nutritional value of foods, some studies have shown the possible antinutritive, adverse physiological, and toxic effects of Maillard browned food products after heat treatment or storage (Lang et al., 1959; Adrian, 1973, 1974; Sgarbieri et al., 1973; Lee et al., 1974, 1977a,b; Tanaka et al., 1977). However, there is no report as to specifically which constituents of Maillard browned products might

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0021-8561/79/1427-0478$01.00/0