Chapter 3

STABLE ISOTOPE BACKGROUND

Terms and notation common to stable isotopic research and used throughout this thesis may be found on page 261. The reader should be familiar with the meaning of stable isotope, isotope fractionation, isotope effects, and the reporting of isotopic compositions for the best understanding of the discussion which follows. Significant information about floral or faunal identity and habitats comes from our understanding of the stable isotopic fractionation processes which occur in the biosphere.

For paleodietary studies, the utility of stable carbon and nitrogen isotopes derives from variation in the isotopic ratios of marine and terrestrial dietary sources. Variation in the isotopic composition of foods derives from fractionation occurring in biochemical or physiological systems, or differences in the isotopic composition of element sources (e.g., seawater versus fresh water). Stable isotopic properties and fractionation processes involved with carbon, nitrogen, and oxygen are relevant because paleodietary specimens are converted to dinitrogen and carbon dioxide gases for mass spectrometric measurements. Thus, the properties, processes, and sources of variation in the isotopic ratios of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^{18}\text{O}/^{16}\text{O}$ are the foundation for interpretation of dietary composition.

Processes involved in the fractionation of stable carbon and nitrogen isotopes include photosynthesis and nitrogen fixation. The reaction, photosynthesis, by which green plants manufacture carbohydrates from carbon dioxide (CO$_2$) and water (H$_2$O) uses sunlight as an energy source and chlorophyll as a catalyst. Three types of photosynthesis (i.e., C$_3$–based, C$_4$–based, or CAM) have been recognized and differ in how the fractionation of stable carbon isotopes occurs. Each type shows a distinctive range of stable isotopic compositions. These differences provide signatures from which diet may be inferred.
Carbon isotope fractionation occurs during biosynthesis and in the uptake and intracellular diffusion of CO$_2$. Within each photosynthetic cycle some variation in isotopic composition exists among different plants depending on biochemical, metabolic, and environmental factors. Theories and models for the control of isotopic discrimination in each photosynthetic pathway are necessary for understanding how variation in isotopic composition occurs and which factors are involved. Progressive isotopic enrichment along food chains, isotopic differences in plants due to climate, temperature, altitude, or the processing of food before consumption contribute to the isotopic composition of dietary items. These variations in composition complicate the interpretation of isotopic measurements in terms of percentages of food items in the diet, comparison of groups across time periods, or comparison of groups from different ecosystems.

On a worldwide basis, plants using C$_4$–based photosynthesis include sorghum, millet, maize, sugar cane, and some other tropical grasses (e.g., some amaranths and Chenopods). C$_3$–based flora include wheat, rice, forest grasses, montane grasses, wetland grasses, root crops, legumes, vegetables, trees, and shrubs. While other plant groups such as succulents (e.g., cacti), epiphytes, euphorbias (e.g., spurge), agaves, and bromeliads (e.g., pineapple) are CAM–based.

**Carbon cycle**

Photosynthesis occurs when CO$_2$ enters the plant through the stomata in the leaves, is fixed by enzymes, and then converted into carbohydrates. The steps within the photosynthetic process result in a fractionation of isotopes. One measure of carbon isotope discrimination by plants is $\Delta$, or the deviation of the isotope effect or fractionation factor from unity (Farquhar, Ehleringer, and Hubick 1989). Thus, $\Delta = \alpha - 1$, where
\[ \alpha = \frac{R_{\text{air}}}{R_{\text{plant}}} \], following the general definition of \( \alpha \) in Appendix D. In terms of standard notation\(^1\)

\[ \Delta = \frac{\delta^{13}C_{\text{air}} - \delta^{13}C_{\text{plant}}}{1000 + \delta^{13}C_{\text{plant}}} \times 1000 \]

where \( \delta^{13}C_{\text{air}} = -8\% \), the value for atmospheric CO\(_2\) in 1988 (Farquhar, Ehleringer, and Hubick 1989).

**Calvin cycle (C\(_3\))**

The photosynthetic carbon reduction cycle (PCR) is the fundamental CO\(_2\) assimilatory process, which is expressed as the reaction:

\[ 3\text{CO}_2 + 9\text{ATP} + 6\text{NADPH} + 5\text{H}^+ \Rightarrow \]

\[ \text{C}_3\text{H}_5\text{O}_3\text{P} + 9\text{ADP} + 8\text{P}_1 + 6\text{NADP}^+ + 3\text{H}_2\text{O} + 468 \text{kJ/mol} \]

where ATP stands for adenosine triphosphate (C\(_{10}\)H\(_{16}\)N\(_5\)O\(_{13}\)P\(_3\)), NAD refers to nicotinamide adenine dinucleotide (a coenzyme that carries hydrogen atoms in electron-transfer reactions), and ADP denotes adenosine diphosphate (C\(_{10}\)H\(_{15}\)N\(_5\)O\(_{10}\)P\(_2\)).

In C\(_3\)-photosynthesis (also known as the Calvin cycle, Calvin–Benson cycle, pentose cycle, or reductive pentose phosphate pathway) 3-phosphoglyceric acid (3PGA), a three carbon compound, is the first product of the cycle (Bender *et al.* 1973, Lawlor 1987). Ribulose bisphosphate carboxylase oxygenase enzyme (Rubisco) reacts with CO\(_2\) to produce phosphoglycerate (Boutton 1991b: 176, Hoefs 1997: 41). Chloroplasts perform most of the work in photosynthesis, including the creation of 3-carbon phosphorylated compounds (Lawlor 1987).

\(^1\) Farquhar, Ehleringer, and Hubick (1989) prefer to exclude the factor of 1000 from calculations, but it is include here for consistency with accepted isotopic measurements in per mil.
A model, proposed by Farquhar, Ehleringer, and Hubick (1989), for discrimination during C₃–photosynthesis incorporates the contributions of differential diffusion across the stomata of ¹²CO₂ versus ¹³CO₂ (denoted as \( a \) and theoretically equal to 4.4‰), the fractionation during the Rubisco reaction (\( b \), determined by experiment to fall between 26.4‰ and 28.5‰), and the ratio of ambient \( (p_a) \) to intercellular \( (p_i) \) CO₂–partial pressures:

\[
\Delta = a + (b - a) \frac{p_i}{p_a}
\]

where \( p_i/p_a \) differs by plant species from 0.3 to 0.85.

**Hatch–Slack cycle (C₄)**

In the C₄–cycle (also termed C₄–photosynthesis, Hatch–Slack cycle, or dicarboxylic acid cycle) additional metabolic systems have evolved for accumulating CO₂ and passing it to the PCR–cycle. This form of assimilating CO₂ can increase the efficiency of photosynthesis, particularly under adverse environmental conditions. Plants utilizing this photosynthetic pathway produce 4–carbon carboxylic acids as the primary product, hence the C₄ designation, by passing assimilated CO₂ into the 3–carbon acid precursor phosphoenol pyruvate carboxylase, or PEP (Bender *et al.* 1973, Hoefs 1997: 41, Lawlor 1987).

Farquhar (1991: 485) suggests that the expression for discrimination in C₄–species be altered from the one for C₃–photosynthesis to account for the first carboxylation by PEP carboxylase¹. The estimated discrimination becomes

\[
\Delta = a + (b_4 + b_3\Phi - a) \frac{p_i}{p_a}
\]

¹ PEP initially fixes HCO₃, not CO₂ (Ehleringer 1991: 191).
where the effective $^{13}\text{C}$–discrimination by PEP carboxylase ($b_4$) is approximately $-5.7\%e$.

The fraction of carbon fixed by PEP ($\phi$) which leaks out of the bundle sheath is often about 0.34 and may be related to anatomical differences. The Rubisco $^{13}\text{C}$–discrimination ($b_3$) is approximately $30\%e$ (Farquhar, Ehleringer, and Hubick 1989).

If the bicarbonate substrate is in isotopic equilibrium with the intracellular gaseous CO$_2$, then the bicarbonate will show a $7.9\%e$ $^{13}\text{C}$–enrichment relative to the CO$_2$. Isotopic variation among C$_4$–plants is less than in C$_3$–plants because of the conversion step involving HCO$_3$ and the increased CO$_2$–partial pressure within the bundle sheath cells (Farquhar, Ehleringer, and Hubick 1989).

**Crassulacean acid metabolism (CAM)**

A third form of photosynthesis, crassulacean acid metabolism (CAM), operates by accumulating CO$_2$ and synthesizing oxaloacetate (OAA) by PEP carboxylase (Farquhar, Ehleringer, and Hubick 1989: 515). CAM plants absorb CO$_2$ at night for malic acid synthesis. OAA is reduced and stored as maltate (Farquhar, Ehleringer, and Hubirck 1989: 515, Lawlor 1987). Many CAM plants engage in direct C$_3$–photosynthesis during the day by decarboxylating maltate and using Rubisco to fix the released CO$_2$. When CAM plants only operate at night they have a $\delta^{13}\text{C}$ value of $-11\%e$, daytime–only plants average $-28\%e$, while most range between $-10\%e$ and $-20\%e$ (like C$_4$–plants) (Lawlor 1987). Differing temperature and lighting levels cause shifts in the $\delta^{13}\text{C}$ values of CAM plants (Bender *et al.* 1973). Carbon dioxide fixation activity alters from C$_3$–dominant to C$_4$–dominant enzymatic activity as the light exposure period shortens (Lerman and Queiroz 1974).
stress or drought will induce a reversible switch in some arid habitat species from C₃ to CAM photosynthesis (Farquhar, Ehleringer, and Hubick 1989).

**Difference among the carbon photosynthetic cycles**

Overall, C₃–plants have average δ¹³C values of −27.1 ± 2‰, while C₄–plants average −13.1 ± 1.2‰ (O’Leary 1988). Therefore, the average discriminations (Δ) are 19.6‰ and 5.2‰, respectively. In particular, *Zea mays* (maize) has a δ¹³C value of −11.6 ± 0.4‰ (*n* = 120 cultivars) and varies by only 0.3‰ over different ploidy levels or strains (O’Leary 1988).

Aquatic plants, with few exceptions, are C₃–based. Their carbon isotopic composition ranges between −11% and −39‰ (Farquhar, Ehleringer, and Hubick 1989). Carbon isotope ratios differences among freshwater and marine plants are due to differences in the isotopic composition of the source carbon – dissolved CO₂ (dissolved inorganic carbon or DIC)¹, bicarbonate, or gaseous CO₂ (Farquhar, Ehleringer, and Hubick 1989).

Additional conditions influence isotopic discrimination by plants, including altitude, canopy effects, sunlight levels, and reactions to soil salinity. These conditions along with species specific variation account for the worldwide isotopic variation observed within plants utilizing the same photosynthetic processes. Within canopy environments, light conditions and altered isotopic composition of CO₂ recycled during soil respiration lead to 3‰ to 5.6‰ decreases with depth between leaves at the top and bottom of canopies (Farquhar, Ehleringer, and Hubick 1989). It has been observed that increases in soil salinity will often create a discrimination decrease, reflecting a change in intercellular CO₂–partial pressure (Farquhar, Ehleringer, and Hubick 1989). C₃–plants show an average 1.2‰

¹ Mook (1968: 155) observed DIC in fresh water to have δ¹³C equal to −12‰, +1‰ for seawater, and between those values for brackish waters.
increase per 1000 m of altitude (Körner, Farquhar, and Roksandic 1988). The change in
discrimination is mainly due to the decrease in CO₂–partial pressures (\(p_a\)) with altitude
(\(e.g., \) 320 μbar at 500 m AMSL to 200 μbar at 4500 m AMSL) (Körner, Farquhar, and
Roksandic 1988).

Such average ecosystem changes could be factors affecting differences in isotopic
results between Lowland and Highland Maya groups. Therefore, measuring isotopic values
of plants and fauna in the region under investigation provides a more secure foundation for
assessment of the contributions to diet by plants using different photosynthetic pathways.
Little work has been done to provide appropriate dietary models which incorporate
ecosystem variability.

**Nitrogen cycle**

The process of nitrogen fixation\(^1\) converts the chemically unreactive atmospheric
dinitrogen (N\(_2\)) into more reactive forms, such as nitrate (NO\(_3^-\)), nitrite (NO\(_2^-\)) or ammonia
(NH\(_3\)). Ammonia can be taken up directly by plants, or oxidized to NO\(_2^-\) and NO\(_3^-\). The
fixation process is usually performed by microorganisms (\(e.g., Rhizobium, Clostridium, or
Azotobacter\) bacteria) whose enzymes convert nitrogen into an absorbable inorganic form.
Once absorbed by plant roots, nitrogen can be assimilated\(^2\) into organic compounds, such
as amino acids, proteins, or nucleic acids (Bray 1983, Lawlor 1987). Another process,
biological mineralization, occurs when organic compounds are decomposed into simple
inorganic nitrogen compounds, such as urea (NH\(_2\)CONH\(_2\)), nitrate, or ammonium ions

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\(^1\) Biological fixation refers to the conversion of atmospheric nitrogen to organic nitrogen compounds by
organisms (Scholten 1991).

\(^2\) Assimilation refers to the process of inorganic nitrogen compounds serving as nutrients for microorganisms
and plants (Scholten 1991).
Nitrogen isotopic fractionation is greater for nitrification (the oxidation of $\text{NH}_4^+$ to $\text{NO}_3^-$) and denitrification (the reduction of $\text{NO}_3^-$ to $\text{N}_2$), but less for biological fixation and mineralization (Delwiche and Stein 1970, Scholten 1991, Wada 1980).

The nitrogenase enzyme system in nitrogen-fixing organisms reduces dinitrogen\(^1\) (Bray 1983, Lawlor 1987, O’Leary 1988). Although higher plants cannot assimilate $\text{N}_2$, photosynthetic bacteria and blue–green algae (e.g., *Nostoc*) reduce $\text{N}_2$ to $\text{NH}_3$ (Lawlor 1987). Leguminous plants (of the *Leguminosae* family – peas, beans, clover, lupin, soybean, and peanut) fix nitrogen symbiotically with *Rhizobium* bacteria. Non–leguminous plants (e.g., tree and shrubs – genera *Alnus*, *Ceanothus*, and *Myrica*) fix nitrogen through other bacteria (Bray 1983). Legumes show nitrogen isotopic compositions close to atmospheric nitrogen, while non–legumes and non–nitrogen–fixing plants acquire their isotopic composition of their growing medium (Delwiche and Stein 1970).

Biologically fixed atmospheric nitrogen is a major source for terrestrial organic matter. Soil organic matter $\delta^{15}\text{N}$ values range between $-3\%e$ and $18\%e$ vs. air, with forest surface soils ranging between $-2\%e$ and $3\%e$ vs. air (Wada, Imaizumi, and Takai 1984). Soil $\delta^{15}\text{N}$ values will depend on accumulation processes and application of fertilizers (Wada, Imaizumi, and Takai 1984). Rain, biological fixation of molecular nitrogen, and terrestrial runoff of dissolved organic and inorganic nitrogen compounds are major contributing sources for oceans (Hoefs 1987: 131, Scholten 1991, Wada, Kadonaga, and Matsuo 1975).

Terrestrial plants generally have nitrogen isotopic compositions close to atmospheric nitrogen because fixation of nitrogen\(^2\) involves little fractionation (Delwiche and Stein

\[ \text{N}_2 + 6\text{H}^+ + 6 \text{e}^- \rightarrow 2\text{NH}_3 \]

\[ \text{N}_2 \rightarrow \text{NH}_4^+ \]

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\(^1\) $\text{N}_2 + 6\text{H}^+ + 6 \text{e}^- \rightarrow 2\text{NH}_3$

\(^2\) Fractionation factor of 0.991–1.008 for $\text{N}_2 \rightarrow \text{NH}_4^+$ (Delwiche and Stein 1970, Wada, Imaizumi, and Takai 1984, Wada 1980).
Marine organisms generally have $\delta^{15}N$–values more positive than terrestrial ones that are derived from dissolved $\text{NO}_3^-$ and $\text{NO}_4^+$, a larger degree of fractionation by denitrification\textsuperscript{1}, and an input of organic materials rich in $^{15}N$ (Scholten 1991, Schwarz and Schoeninger 1991: 304, Sweeney and Kaplan 1980, Wada 1980, Wada, Kadonaga, and Matsuo 1975). Marine biogenic nitrogen has an average $\delta^{15}N$–value of 7‰ and increases along the food chain to about 20‰ (Hoefs 1987, Wada 1980). Thus, terrestrial nitrogen isotopic values will range between 0‰ and near 10‰, while marine sources will span between 7‰ and 20‰, providing a valuable signature for sourcing foods in human subsistence systems.

**Relationship Between Diet and Tissue Isotopic Composition**

Establishing the association between the isotopic composition of food and the consumer’s tissues is critical for the reconstruction of diet. Accounting for metabolic fractionation must occur before converting isotopic measurements into dietary percentage estimates.

Fractionation during metabolism of food or the incremental difference between diet and bone collagen, denoted as $\Delta^{13}C_{\text{diet-collagen}}$, is often estimated as +5‰, sometimes less (Ambrose and Norr 1993: Table 2). Natural variation in $\Delta^{13}C_{\text{diet-collagen}}$ has been observed at +5‰ for animals feeding on $C_3$–based plants and +6‰ for animals with $C_4$–based diets (van der Merwe 1989: 114). The isotopic fractionation observed in nature between diet and collagen has become more accepted relative to observations from laboratory feeding experiments, in part because of the different foods consumed, the small body size of

\textsuperscript{1} Fractionation factor of 1.02 or greater for $\text{NO}_3^- \rightarrow \text{N}_2$ (Delwiche and Stein 1970, Wada 1980).
laboratory animals, and the metabolic differences between laboratory and wild animals (Ambrose and Norr 1993).

For mice raised on diets of known carbon isotopic composition (Table 3.1) an average fractionation of 3.9‰ was observed with animals raised on the same diet showing equivalent results (DeNiro and Epstein 1978, DeNiro and Epstein 1981). However, isotopically different diets seem to result in different collagen–to–diet spacing\(^1\). The same mice had an approximate \(\Delta^{13}C_{\text{diet-collagen}}\) of 9.6‰ in the isotopic composition of their bone bicarbonate, regardless of the isotopic composition of the diet. For mice raised on diets of known nitrogen isotopic composition (Table 3.2) an average fractionation of +2.4‰ was observed (DeNiro and Epstein 1981).

Pigs, which are digestively and metabolically more similar to humans than mice, were raised on diets of 100% C\(_3\)–based or 100% C\(_4\)–based foods (Hare et al. 1991). The results indicate again that \(\Delta^{13}C_{\text{diet-collagen}}\) varies slightly according to the carbon isotopic composition of the experimental diet (Table 3.1 and 3.2). Glycine, which composes 33% of collagen, showed little isotopic difference between diet and its incorporation into bone collagen, but dietary glycine was 6–8‰ enriched in \(^{13}\)C relative to the bulk diets. Thus it was concluded that glycine contributed to the enriched signal in bone collagen (Hare et al. 1991).

For 15 minks raised on the same diet and three rabbits fed a monotonous diet, the mean stable carbon and nitrogen isotopic values of bone collagen from different skeletal elements was found to be statistically insignificant\(^2\) (DeNiro and Schoeninger 1983). Differences between males and females were also statistically insignificant. The inference from these

\(^1\) collagen for diet no. 1 = −22.3‰ + 4.4‰ or −17.9‰, collagen for diet no. 2 = −18.3‰ + 3.5 or −14.8‰, collagen for diet no. 3 = −19.3‰ + 3.8 or −15.5‰.

\(^2\) The greatest isotopic differences were for minks a \(\Delta^{13}C_{\text{collagen-diet}}\) value of 0.6‰ and 0.7‰ for \(\Delta^{15}N_{\text{collagen-diet}}\), while for rabbits 0.3‰ for both \(\delta^{13}C\) and \(\delta^{15}N\).
results is that collagen has the same isotopic composition regardless of the sex of the individual or the skeletal element from which collagen is extracted.

Animal–to–diet fractionation was observed for two species of amphipods raised on two diets of similar isotopic composition. Average isotope fractionation for two amphipod species relative to diet Amphithoe valida was −0.3‰ for $\Delta^{15}N_{\text{tissue-diet}}$ and −0.7‰ for $\Delta^{13}C_{\text{tissue-diet}}$, while Parhyale hawaiensis was −0.9‰ for $\Delta^{13}C_{\text{tissue-diet}}$ and 2.4‰ for $\Delta^{15}N_{\text{tissue-diet}}$, and was fairly consistent regardless of the diet consumed (Macko, Lee, and Parker 1982). The authors concluded that differences were species based, rather than dietary.

Enrichment of $^{15}N$ in animal tissues occurs from lower to higher trophic levels in both terrestrial and marine food chains (Minagawa and Wada 1984, Schoeninger, DeNiro, and Tauber 1983, Wada 1980). A result consistent with the observation that $\Delta^{15}N_{\text{diet-animal}}$ fractionation is between 1–5‰ depending on diet, species, and the body tissue that was measured. Marine animals show a $\Delta^{15}N_{\text{diet-animal}}$ between 2–5‰ (Minagawa and Wada 1984).

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1. The isotopic composition of the diets were for $\delta^{13}C$ $-14.6‰$ and $-14.3‰$ and for $\delta^{15}N$ $8.1‰$ and $7.9‰$. 
### Table 3.1: Stable carbon isotopic ratio fractionation between diet and collagen

<table>
<thead>
<tr>
<th>specimen</th>
<th>$\delta^{13}\text{C}_{\text{diet}}$</th>
<th>$\Delta^{13}\text{C}_{\text{collagen-diet}}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>mice$^a$</td>
<td>$-22.3 \pm 0.5%$</td>
<td>4.4%$^b$</td>
<td>10</td>
</tr>
<tr>
<td>mice$^a$</td>
<td>$-18.3 \pm 0.6%$</td>
<td>3.5%</td>
<td>4</td>
</tr>
<tr>
<td>mice$^a$</td>
<td>$-19.3% \pm 0.4%$</td>
<td>3.8%$^c$</td>
<td>4</td>
</tr>
<tr>
<td>pigs$^d$</td>
<td>$-12.4%$</td>
<td>3.2%</td>
<td>10</td>
</tr>
<tr>
<td>pigs$^d$</td>
<td>$-25.3%$</td>
<td>1.4%</td>
<td>10</td>
</tr>
</tbody>
</table>

b. Reported as 3.7‰ in DeNiro and Epstein 1978, but was changed to 4.4‰ because collagen extraction methods changed in DeNiro and Epstein 1981.
c. Reported as 2.8‰ in DeNiro and Epstein 1978, but was changed to 3.8‰ because collagen extraction methods changed in DeNiro and Epstein 1981.
d. Hare et al. 1991.
e. 100% C₄–based diet.
f. 100% C₃–based diet.

### Table 3.2: Stable nitrogen isotopic ratio fractionation between diet and collagen

<table>
<thead>
<tr>
<th>specimen</th>
<th>$\delta^{15}\text{N}_{\text{diet}}$</th>
<th>$\Delta^{15}\text{N}_{\text{collagen-diet}}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>mice$^a$</td>
<td>4.6 $\pm$ 0.3‰</td>
<td>3.4‰</td>
<td>10</td>
</tr>
<tr>
<td>mice$^a$</td>
<td>5.8 $\pm$ 0.3‰</td>
<td>1.4‰</td>
<td>4</td>
</tr>
<tr>
<td>mice$^a$</td>
<td>5.1 $\pm$ 0.7‰</td>
<td>2.5‰</td>
<td>4</td>
</tr>
<tr>
<td>pigs$^b$</td>
<td>3.2‰</td>
<td>2.3‰</td>
<td>10</td>
</tr>
<tr>
<td>pigs$^b$</td>
<td>1.8‰</td>
<td>2.2‰</td>
<td>10</td>
</tr>
</tbody>
</table>

a. DeNiro and Epstein 1981
b. Hare et al. 1991.
Sutoh, Koyama and Yoneyama (1987) found that \( \delta^{15}N \)-values of milk from Holstein cows were consistently enriched by 2\( \% \) relative to diet. This is consistent with a \( \Delta^{15}N_{\text{fingernails-diet}} \) of 2–3\( \% \) observed in nursing infants relative to their weaned cohorts (Fogel, Tuross, and Owsley 1989).

**Isotopic Alteration Due to Food Preparation Techniques**

Food preparation (e.g., cooking, grinding, soaking, and fermentation) induce changes to food including the aspects of flavor, texture, digestibility, and nutritional value (Coe 1994). Therefore, it becomes important to know the isotopic composition of foods before and after processing them. Isotopic fractionation due to food processing before consumption has been examined for a few plants commonly eaten by the ancient Maya and other American peoples. The effects of boiling, roasting, liming, molding, fermentation, and carbonization on the stable isotopic composition of *Zea mays* cobs, *Helianthus annuus* seeds, *Agava americana* leaves, and *Pachyrrhizus erosus* tubers was explored in laboratory experiments (Marino and DeNiro 1987). The stable isotopic ratios of carbon, hydrogen, and oxygen in the plant samples showed no statistically significant alteration from *in vivo* values, except for the *Agave americana* leaves (Marino and DeNiro 1987). The authors concluded that food preparation has little or no effect on isotopic ratios of plants from the three major photosynthetic groups (Marino and DeNiro 1987).

Wright (1994: 219-226) tested for altered \( \delta^{13}C \)-values due to alkaline (lime) processing of maize, a simulation of *nixtamalization*. Yellow, black, and white maize kernel samples were treated by boiling and soaking in a lime solution and in plain water. No significant differences were observed in isotopic composition among the processed and untreated maize samples (Wright 1994: 223-224).
Thus, food processing methods appear to have little effect on isotopic composition. Whether plants are consumed untreated or in a processed form, the original isotopic composition can be expected to be retained and transferred to the consumer.