Age-Specific Changes of Polypeptides in Peanut Leaves

Proteins from peanut leaves at five stages of maturity were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Two fractions of differing solubilites were extracted from each leaf—buffer- and detergent-soluble fractions. Although many polypeptide bands were common to all leaf ages in both extraction media, electrophoretic patterns of buffer-soluble proteins differed from those of detergent-soluble proteins. Many changes occurred in the molecular weight distribution of buffer-soluble proteins with aging, but fewer changes occurred with aging in the molecular weight distribution of polypeptides solubilized by detergent. Proteins associated with aging could be classified into three groups: those more prominent in young leaves, those more prominent in old leaves, and those equally prominent at all ages studied.

Several authors (Betschart and Kinsella, 1973; Edwards et al., 1975; Neucere and Godshall, 1979) have suggested that leaf protein concentrate be incorporated into the human diet. The quality of extracts from the mature peanut leaf have been shown to be sufficiently high for use as a dietary supplement in conventional foods (Neucere and Godshall, 1979). Since physiological and biochemical parameters have been analyzed in plants and shown to vary with leaf age, the value of leaf protein concentrate as a food additive may also vary with leaf age. The photosynthetic capacity of the individual peanut leaf has been shown to decrease after it is 3–4 weeks old (Pallas and Samish, 1974); thus polypeptides may also change at this age. Downton and Slatyer (1971) found leaf age in *Atriplex* to be a source of variation in the level of some enzymes when expressed on a fresh weight basis but not when expressed on a protein basis. The membrane proteins associated with chloroplast maturation in corn (*Zea mays*) have been characterized and shown to change with the greening of leaves (Grebanier et al., 1979). The objective of this study was to characterize the polypeptide components from different soluble fractions of peanut leaves at various stages of maturity. Such information should aid in the evaluation of peanut leaves as a dietary supplement by providing information on the extractability and variability of proteins as related to leaf age.

EXPERIMENTAL SECTION

Leaves. Peanut plants (*Arachis hypogaea* L., cv. "Florunner") were grown for approximately 5 weeks after germination in a growth chamber as described previously (Pallas and Samish, 1974). Leaves were collected from the primary upright stem. Samples were taken from the youngest leaf at the apex to the oldest leaf at the base. The apical folded leaf was designated leaf 0. The youngest unfolded leaf was designated leaf 1 and was approximately 1 week postemergent. Leaf 2 was approximately 2 weeks postemergent, leaf 3, 3 weeks postemergent, and leaf 4, 4 weeks postemergent. The data in this study are based on samples taken from two plants of the same age on three different dates; i.e., the results for each leaf age are based on the analyses of six leaves. After leaf weight was determined, leaves were cut into 1-mm sections and stored in liquid nitrogen until used.

Extraction Procedure. Leaves were homogenized at 4 °C in a Kontes Duall tissue grinder using a buffer containing a protective agent for –SH groups, salts, and protease inhibitors. The composition of this buffer was 0.01 M Tris-HCl, pH 7.5, 5 mM dithiothreitol, 25 mM $MgCl_2$, 25 mM KCl, and 1 mM L-1-tosylamido-2-phenylethyl chloromethyl ketone, and 1 mM phenylmethanesulfonyl fluoride. The homogenate was centrifuged at 12000*g* for 20 min at 4 °C. The supernatant was decanted and designated buffer soluble (BS). An aliquot of BS was removed for protein determination by the method of Bradford (1976). The remainder of the BS supernatant was made 10% glycerol, 2% $NaDodSO_4$ and 5% mercaptoethanol, heated to 100 °C for 10 min in a Tecam Dri-Block, and stored at −20 °C. The pellet from the 12000*g* centrifugation above was then suspended in the starting buffer with the addition of glycerol, $NaDodSO_4$, and mercaptoethanol so that the final concentration was 10%:2%:5%, respectively. This suspension was allowed to stand at room temperature for 1 h with intermittent agitation by rotating the sample tube by hand. The suspension was then centrifuged at 12000*g* for 20 min at 18 °C. The supernatant was decanted and designated detergent soluble (DS). This fraction was also heated at 100 °C for 10 min in a Tecam Dri-Block and stored at −20 °C. Consequently, on each collection date, 20 samples were examined—5 different age leaves with 2 replicates of each age were taken, making a total of 10 leaves. From each leaf 2 fractions of differing solubility (buffer and detergent soluble) were extracted, accounting for a total of 20 samples.

Electrophoresis. Leaf extracts were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis using the buffer system of Laemmli (1970): 0.125 M Tris-HCl, pH 6.8, in the stacking gel; 0.375 M Tris-HCl, pH 8.8, in the separating gel; 0.025 M Tris with 0.192 M glycine, pH 8.3, in the electrode buffer. Electrophoresis was performed on 0.75 mm thick slab gels with a 12.5% polyacrylamide separating gel (16 cm high and 14 cm wide) and a 1 cm high stacking gel. Gels were prerun for 30 min at a current of 10 mA before samples were applied. The proteins used as molecular weight markers were phosphorylase *b* (94 000), bovine serum albumin (68 000), ovalalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (21 000), and lysozyme (14 300) (Bio-Rad Laboratories sodium docecyl sulfate–polyacrylamide gel electrophoresis standards). Electrophoresis was conducted at 21 °C with a current of 10 mA/gel for 1 h and then of 18 mA/gel for 2.5 h. Gels were stained by the silver technique of Switzer et al. (1979).

RESULTS AND DISCUSSION

The total amount of BS protein per leaf varied with leaf age (Figure 1). The youngest leaf (leaf 0) had the least amount of BS protein when expressed on a per leaf basis; however, leaf 0 had the greatest amount of BS protein when expressed on a gram fresh weight basis. On a per leaf basis, the first and second unfolded leaves (leaf positions 1 and 2) had the greatest amount of BS protein while leaves in positions 3 and 4 had greatly decreased BS protein. When expressed as milligrams of BS protein per gram fresh weight, protein decreased with increasing leaf age down to leaf position 3 with the amounts in leaves 3

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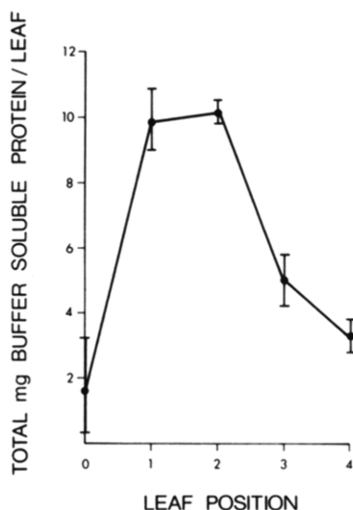

Figure 1. Total buffer-soluble protein in each leaf as a function of leaf position. Leaf positions 0 and 4 represent the youngest leaf and oldest leaf, respectively.

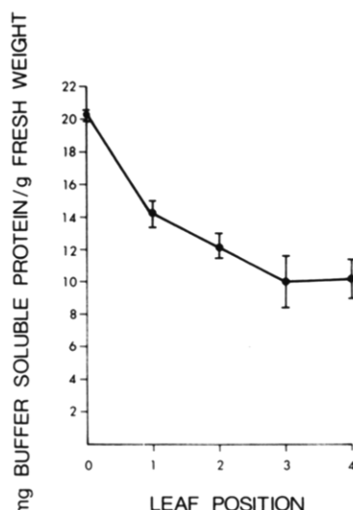

Figure 2. Milligrams of buffer-soluble protein per gram fresh weight as a function of leaf position. Leaf positions 0 and 4 represent the youngest leaf and oldest leaf, respectively.

and 4 remaining fairly constant (Figure 2).

Since an equal amount of leaf tissue extract based on fresh weight was applied to each lane of the polyacrylamide gels in Figures 3 and 4, the differential banding patterns suggest the BS protein concentration of leaf 0 was greater than the other leaves (Figure 3). This concept is supported by the data in Figure 2 demonstrating the protein concentrations in the leaves. Likewise, there appear to be greater DS protein concentrations based on leaf fresh weight in leaves 3 and 4 than for the younger leaves (Figure 4). This possibly indicates increased synthesis of detergent-soluble components, such as thylakoid membranes, with increasing age. Since compounds used in the extraction of the DS fraction interfere with protein analysis, protein determinations could not be made for this fraction.

Even though there were qualitative differences in the polypeptides of the BS fraction with respect to leaf age, the majority of the bands are present at all ages studied (Figure 3). Many of the differences represent components that are most abundant in the older leaves, including polypeptides of the molecular weights 102 000, 101 500, 100 500, 98 000, 55 000, 42 500, 26 000, and 14 000. The bands of 55 000 and 14 000 molecular weights are possibly the large and small subunits of ribulosebisphosphate

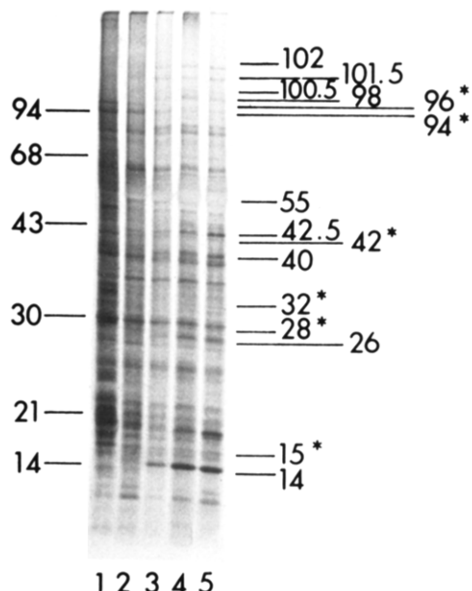

Figure 3. NaDodSO$_4$–polyacrylamide gel electrophoresis of water-soluble proteins extracted from peanut leaves. Lanes 1, 2, 3, 4, and 5 are leaf positions 0, 1, 2, 3, and 4, respectively. The numbers to the left are the molecular weight standards × 10^{-3} and are positioned at the point to which they migrated. The numbers to the extreme right with an asterisk are the molecular weights of polypeptides more evident in younger leaves; those without an asterisk are the molecular weights of polypeptides more evident in older leaves.

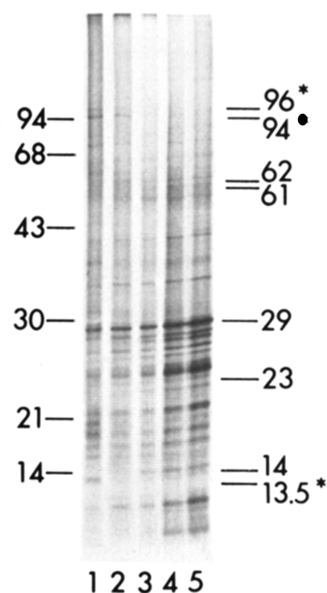

Figure 4. NaDodSO$_4$–polyacrylamide gel electrophoresis of detergent-soluble proteins extracted from peanut leaves. See the legend to Figure 3 for the significance of the number designations.

carboxylase (RuBPCase). These are the molecular weights reported for RuBPCase subunits in the pea plant, *Pisum sativum* (Highfield and Ellis, 1978), which is closely related to the peanut. The youngest peanut leaf showed little if any of these subunits in either the BS or DS fraction. These results can be related to the work of Pallas and Samish (1974), who found the photosynthetic rate of 2–4-week-old leaves to remain rather constant.

There are several bands which are more evident in the BS fraction of young leaves than in old leaves when compared to bands equally distinct at all ages (Figure 3). These have molecular weights of 96 000, 94 000, 42 000, 32 000, 28 000, and 15 000. Two of these bands 96 000 and

94 000 are more evident in the DS fraction of young leaves than in that of old leaves (Figure 4). Overall, there are fewer differences in young leaves and old leaves of the DS fraction than in those of the BS fraction. In addition to the 96 000 and 94 000 molecular weight bands, band 13 500 is most prominent in young leaves. The bands most distinct in the DS fraction of older leaves have the molecular weights of 61 000, 62 000, and 14 000, and the entire complex of bands in the molecular weight range of 23 000–29 000. This complex of proteins may be part of the light harvesting complex (LHC) of chloroplast thylakoid membranes which has been identified as a group of proteins within this molecular weight range in a wide variety of plants (Grebanier et al., 1979).

From the results, it is apparent that the overall leaf protein composition changes very little with age of the leaf, but some age-specific changes in the polypeptide profiles occur. These data should be considered in evaluation of peanut leaf protein for human consumption; likewise, the leaf age may affect the quality of the leaf protein concentrate in other plant species. The nutritional value of the proteins obtained from different age leaves and cellular fractions should be analyzed and used in determining processing methods of peanut leaves as a dietary supplement.

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Mass Spectra and Sensory Properties of Some 4,5-Dialkyloxazoles

Fifteen 4,5-dialkyloxazoles have been synthesized by the reaction of corresponding bromo ketones with formamide. Mass spectra data and preliminary odor descriptions of synthesized 4,5-dialkyloxazoles are given. The general molecular structure for bell pepper like aroma compounds proposed by Buttery et al. could be extended to 4,5-dialkyloxazoles.

Among about 3000 known constituents of aroma (Ohloff and Flament, 1978), heterocyclic compounds deserve particular attention. Due to their characteristic odor, heterocyclic compounds contribute significantly to the flavor of processed foods. Alkyloxazoles are heterocyclic compounds containing both nitrogen and oxygen. The report of Stoffelsma and Pypker (1968) was the first identifying an oxazole as naturally occurring in a food. Since then, alkyloxazoles have been found in several foods, including roasted coffee (Stoffelsma et al., 1968; Vitzthum and Werkhoff, 1974), roasted cocoa (Vitzthum et al., 1975), roasted barley (Harding et al., 1978), baked potato (Coleman et al., 1981), roasted peanut (Lee et al., 1981), and meat products (Chang and Peterson, 1977; Mussinan and Walradt, 1974).

The published mass spectra data of oxazoles are very limited (Bowie et al., 1968; Vitzthum and Werkhoff, 1974). In this paper, mass spectra and sensory properties of 15 4,5-dialkyloxazoles, which have not yet been found in foods, are reported.

EXPERIMENTAL SECTION

Materials. Aliphatic ketones, bromine, and formamide were obtained from reliable sources.

Bromo Ketones. The method used was that of Catch et al. (1948). In most cases where two isomeric bromo ketones were formed, they were not separated but taken through the oxazole synthesis, and the isomeric oxazoles were separated by gas–liquid chromatography.

4,5-Dialkyloxazoles. These were all synthesized by the general method of Lindberg et al. (1970). The bromo ketone was allowed to react with formamide. Yields were all satisfactory at about 50–60%. In the cases where a mixture of two isomeric bromo ketones was used, the isomeric 4,5-dialkyloxazoles were separated by GLC using a 12 ft. long × $^1/_8$ in. o.d. stainless steel column packed

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