Electrophoretic Observations on Protein Changes and Variability during Development of the Peanut

In gel electrophoretic patterns of extracts from whole peanuts (*Arachis hypogaea* L.) bands of large molecular weight storage globulins intensified during fruit development and became diffuse when germination was initiated. Intravarietal protein (*e.g.*, arachin) polymorphism that occurred among mature peanuts of several cultivars grown in different areas of the United States was not related to any of the changing patterns of developing peanuts. This indicated that physiologically immature or "overmature" seeds were not accidentally included in the samples considered mature in these studies. However, mature Oklahoma-grown Spanish peanuts produced banding patterns resembling those of immature seeds from Georgia-grown peanuts indicating that both contained low concentrations of large molecular weight storage globulins.

Research on seed protein biosynthesis and variability during the different stages of development, maturation, and germination has contributed to a better understanding of the genetic and biochemical mechanisms associated with protein quality and quantity (Aldana *et al.*, 1972; Hall *et al.*, 1972; Kamra, 1971; Juo and Stotzky, 1970; and Nelson, 1969). Polyacrylamide and starch gel electrophoretic techniques were employed to develop and characterize "standard" patterns and zymograms of seeds from a number of commercial peanut varieties of *Arachis hypogaea* (Cherry and Ory, 1973a,b; Cherry *et al.*, 1971, 1972, 1973; Ory and Cherry, 1972). An extensive electrophoretic examination of these peanut proteins from many individual seeds showed much intravarietal polymorphism which was consistent among all of the cultivars (Cherry *et al.*, 1971). In addition, qualitative and semiquantitative protein differences on electrophoretic gels distinguished some of the peanut types grown in different geographical locations. This communication reports the changes in gel electrophoretic patterns of proteins of peanuts at different stages of development and further attempts to determine if seeds of apparently similar maturity in various samples have different electrophoretic patterns.

MATERIALS AND METHODS

Biological materials used for these experiments are summarized in the legend of Figure 1. Immature seeds of Georgia-grown Florigiant, screen-sorted into average sizes and weights as follows [(a) 0.48 × 1.22 cm, 0.12 g; (b) 0.50 × 1.32 cm, 0.20 g; (c) 0.58 × 1.38 cm, 0.29 g; and (d) 0.64–0.96 × 1.41–2.00 cm, 0.36–0.76 g] were arbitrarily categorized into age groups 3, 6, 9, and 12 weeks, respectively (Cherry and Ory, 1973a,b). Protein isolation and polyacrylamide disc gel electrophoretic techniques were described by Cherry *et al.* (1971, 1973) and Cherry and Ory (1973a,b).

RESULTS AND DISCUSSION

Anatomical and biochemical changes (*i.e.*, mostly quantitative observations on storage metabolites) during peanut development have been reported earlier (Aldana *et al.*, 1972; Young *et al.*, 1972; Schenk, 1961). Figure 1 (B, C, and D) illustrates changes (qualitative and semiquantitative) observed in gel electrophoretic patterns of proteins from immature, mature, and germinating seeds of Georgia-grown cultivars (*e.g.*, Florigiant) that can be correlated with stages of development. Immature seeds from Florigiant were used in this study because of their availability. Furthermore, samples of mature seeds of all peanut cultivars (Spanish botanical and Virginia botanical and market types) examined in this study and collected from different locations (except Oklahoma) consistently showed similar protein polymorphism (Figure 1C; Cherry *et al.*, 1971). Therefore, it was assumed that their protein patterns during development would be similar and that Florigiant could be used as a representative cultivar.

Immature peanuts estimated to have developed 3–12 weeks after pegging were examined. Intense biochemical activity (*i.e.*, synthesis and storage of metabolites) and an increase in seed size are typical of this period of development (Aldana *et al.*, 1972; Schenk, 1961). The protein profiles during this period of biochemical activity revealed a rapid increase in the large molecular weight globulins (*e.g.*, arachin) in region 1.0–2.5 cm as the seeds approached maturity (Figure 1B). After harvesting and during drying of mature peanuts, growth and metabolic activity decelerated (Aldana *et al.*, 1972; Schenk, 1961). During this period the globulins in region 1.0–2.5 cm became more prevalent with a corresponding decrease of low molecular weight proteins in region 4.0–7.0 cm (Figure 1B,C). Mature peanuts imbibed for 24 hr to determine the patterns of "overmature" seeds produced diffuse bands in the region of the gel where the large molecular weight globulin, arachin, was normally located (Figure 1D). These protein patterns of immature, mature, and germinating peanuts further supported our earlier studies on dormant seeds suggesting that physiologically immature peanuts were not accidentally included in the samples considered mature and, therefore, the protein polymorphism observed was attributed to genetic and/or environmental factors (Cherry and Ory, 1973a,b; Cherry *et al.*, 1971, 1972, 1973; Ory and Cherry, 1972).

On the other hand, Oklahoma-grown Spanish peanuts were shown to contain fewer large molecular weight globulins than seeds grown in other areas (Cherry *et al.*, 1971). Moreover, these protein patterns resembled those of immature (approximately 3–4 weeks after pegging) Georgia-grown Florigiant peanuts (Figure 1a,b). This was especially evident in the upper half of the gels which contained low concentrations of arachin and the lower portion showed distinct bands of low molecular weight proteins. These data suggested that the Oklahoma-grown Spanish peanuts examined in our studies and considered mature at the time of harvesting may not have reached the same level of maturity and subsequent protein synthesis and storage as peanuts examined from other areas in the United States.

The exact agronomic conditions (*e.g.*, soil types, nutrient levels, and weather) during the growing period of these peanuts along with harvesting and curing data were not carefully determined for this study. In a quantitative study of protein and oil content of 26 strains and cultivars of Georgia-grown peanuts over an 8-year period, it was observed that there were extreme seasonal effects on both constituents, some of which were due to varying climatic conditions (Holley and Hammons, 1968). Consequently, when genetic or chemical analyses (*e.g.*, using gel electrophoretic techniques) of peanuts are used for quality determinations of proteins during the development of new or

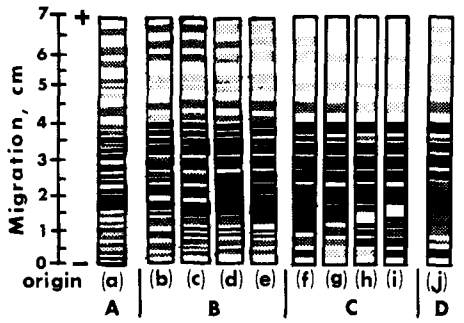

Figure 1. Diagrams of disc gel electrophoretic patterns of proteins from immature, mature, and germinating peanuts: (A) gel a, representative pattern of mature peanuts of Oklahoma-grown cultivars (*A. hypogaea* L. subsp. *fastigiata* var. vulgaris; Spanish botanical type; Comet, Starr, Argentine, or Spanhoma); (B) gels b–e, patterns of immature peanuts of Georgia-grown Florigiant (subsp. *hypogaea* var. hypogaea; Virginia botanical, Virginia market type) categorized into age groups 3 (b), 6 (c), 9 (d) and 12 (e) weeks; (C) gels f–i, four patterns that are representative of peanuts from Florigiant and also other cultivars (Spanish botanical type: Tifspan, Starr, Argentine, Spancross, and Comet; Virginia botanical, Virginia market type: Virginia 56R, Virginia 61R, NC17, NC5, and NC2; and Runner market type: Early Runner, Florunner, Virginia Bunch 67) grown in one to four locations (Virginia, Georgia, Louisiana, and Texas); (D) gel j, pattern of germinating (24 hr) peanuts of Georgia-grown Florigiant.

improved cultivars with greater stability characteristics and more desirable patterns of nutrient components, it would be desirable to observe these factors closely. Such studies were initiated on the fatty acid composition of peanuts (Young *et al.*, 1972; Worthington *et al.*, 1972; Worthington and Hammons, 1971). Currently, similar studies involving proteins are under investigation in our laboratory.

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Production of 4-Hydroxy-5-methyl-3(2*H*)-furanone, a Component of Beef Flavor, from a 1-Amino-1-deoxy-D-fructuronic Acid

4-Hydroxy-5-methyl-3(2*H*)-furanone (a constituent of beef broth) is shown to be produced from 1-deoxy-1-dibenzylamino-D-fructuronic acid, which in turn was synthesized from the amine and D-glucuronic acid. The furanone can be obtained directly in the crystalline state by chloroform extraction of a reaction solution and was identified from its elemental analysis, infrared, ultraviolet, nuclear magnetic resonance, and mass spectra, as well as by comparison with authentic samples.

4-Hydroxy-5-methyl-3(2*H*)-furanone has been prepared in low yields by heating D-xylose (Severin and Seilmeier, 1968), D-ribose (Peer *et al.*, 1968a), and D-ribose 5-phosphate (Peer *et al.*, 1968b) with amine salts. The compound has a caramel-like or burnt aroma and its isolation from beef broth (Tonsbeek *et al.*, 1968) indicates that it is a component of the odor and flavor associated with cooked beef. From the known facts concerning such reactions (Hodge *et al.*, 1972), as well as the reaction conditions used in its preparation, it can be concluded that this furanone, as well as many other flavor and aroma constituents, is probably produced *via* a reaction involving condensation of an aldose with an amine to produce a 1-amino-1-deoxy-2-ketose (Amadori product) which subsequently dehydrates, with amine elimination, to give the desired product. It is noteworthy in this respect that the decomposition of Amadori products derived from many sugars which are normally found in foods has not been studied.

We have isolated and identified the title compound, which is produced, presumably as a decarboxylation product, from 1-deoxy-1-dibenzylamino-D-fructuronic acid which was prepared from dibenzylamine and sodium D-glucuronate (Heyns and Baltes, 1960).