Composition Changes of Peanut Fruit Parts During Maturation[1]

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ABSTRACT

Studies are reported which follow the changes in starch, sugar, fresh and extraction residue weights of peanut fruit parts during maturation. Observations are also reported on the lipid content of the seed. Starch and sugar contents reached maximum levels first in the pericarp (hull) and then the seed coat (testa). Starch maxima occurred at early and middle maturity (stages 3 [10 mg] and 7 [10 mg]) and sugar maxima at a stage approaching middle maturity and at middle maturity (stage 6 [47 mg] and 8[9 mg]), respectively. In the seed, starch reached a maximum just beyond middle maturity (stage 9 [55 mg]) and then remained constant. Sugar content increased throughout maturation and lipid content became maximum at full maturity (stage 13 [415 mg]) then declined to 385 mg at over-maturity (stage 15). The role of regulating substrate supply to the developing seed seemed to shift from the pericarp to the seed coat with increasing maturity. Observed increases in fruit residue weight suggest that the pericarp is competing with the seed for metabolic resources during the late maturity stages.

Key words: maturation, sugar, starch, lipid, pericarp, testa, seed, **Arachis hypogaea L.**

Studies of changes in enzyme activity levels, protein, nucleic acids, lipids, arginine, sucrose, and seed volatiles during maturation of peanuts have recently been reported (Aldana, *et al.*, 1972; Mason *et al.*, 1969; Pattee, *et al.*, 1970; Rudd and Fites, 1972; Worthington, 1968). These studies, as well as earlier work on peanut seed composition (Pickett, 1950 and Schenk, 1961), dealt primarily with the kernel. Schenk (1961) however, included data on fresh weight, dry weight, and crude protein changes in the peanut fruit parts during maturation. Worthington (1968) studied changes in dry weight, crude lipid content and fatty acid composition of crude lipid and triglyceride fractions at four stages of development of the pericarp, testa, cotyledon, and embryonic axis. Additional information on interrelated compositional changes of the peanut fruit parts with maturity is therefore needed to understand the supportive role of the various fruit parts to the ultimate development of the seed. Since the seed is used for edible purposes additional information about changes in associated fruit parts with maturation may provide further insight into how the seed's edible quality and potential processing quality may be influenced by these associated fruit parts.

Due to difficulties encountered in designating sub-parts of the complete peanut fruit using terms available in the peanut literature it would seem desirable to define the various parts as used in this paper.

Fruit—ovary and its contents.

Pericarp (Hull)—includes epidermis or periderm depending on maturity stage, mesocarp and endocarp.

Seed coat (skin)—testa; primarily the outer integument but may include endosperm, nucellus and inner integument during initial separation stages.

Seed—embryo; embryonic axis and cotyledons.

See Esau (1960); Brennan (1969); Prakash (1960); Smith (1956).

Determination of the physiological age of the seed has been a major obstacle in the study of peanut seed maturation. The peanut plant is indeterminate in growth habit. Consequently seed found on a plant at any one of several harvest dates will span a broad range of developmental stages. For determining kernel (seed plus seed coat) maturity Pickett (1950) suggested the use of a combination of kernel texture, color, tightness of the kernel in the hull, degree of fleshy material, and change of color on the inner side of the hull as the most reliable and simple method. Following this suggestion Schenk (1961) outlined pictorially a weekly maturation scheme for peanut kernels. Pattee, *et al.* (1970) extended this method by describing physical and morphological characteristics used to judge peanut kernel maturity on a weekly basis. It was subsequently found that the characteristics used by Pattee, *et al.* (1970) described physiological stages of maturity rather than a weekly progression of development.

This study was undertaken to provide information on peanut fruit part compositional changes at selected physiological stages during maturation. This information extends available information on peanut fruit composition and points out the need for more definitive work on maturation with relation to peanut quality rather than the general bulk composition work done on "immature" and "mature" classifications of farmer stock peanuts. The effect of sampling date on similar physiological maturity stages during the growing season was determined and the similarity in composition shown.

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Materials and Methods

During 1971, 12 randomly selected complete peanut plants, variety NC-2, were harvested on four dates, July 13, Aug. 4, 30, and Sept. 20, from a peanut field at the Central Crops Research Station, Clayton, North Carolina. Twenty-four hours before harvest the plants were subjected to a $^{14}CO_2$ treatment as described by Pattee et al. (1974). At harvest the plants were pulled, packed in ice, and transported to the laboratory.

The fruit were washed with tap water to remove soil, opened, and classified as to maturity stage (Table 1) according to criteria modified from Pattee, et al. (1970). The fruit were then separated into pericarp, seed coat (testa), and seed and these extracted by the procedure shown in Figure 1. Preliminary tests indicated that, because of the high lipid content of peanut seeds, an initial extraction with chloroform: methanol (2:1) gave the best results. The sugar content of the "crude ethanol-soluble fraction" (Figure 1) was comparable to that obtained when the samples were initially extracted with 80% ethanol. Starch was extracted by the method of McCready et al. (1950) except that the initial water extract of the residue was treated at 100° C for 30 minutes to gelatinize the starch (Pucher et al., 1948). Sugar and starch contents were determined colorimetrically by the method of Doubois et al. (1956) using sucrose and soluble starch, respectively, as the standards. Standard curves were prepared daily.

Table 1. Peanut characteristics used to estimate kernel age.[a]

STAGE	PERICARP	KERNEL
1	Peg swelling commenced, maximum length 1 cm, torpedo shaped.	
2	Torpedo shaped, basel section commencing rapid enlargement, maximum length 1.75 cm.	
3	Enlarging basel section, enlargement of apical section, maximum length 3 cm	
	Very watery, easily smaxhed	Very small held tightly by parenchyma tissue difficult to remove.
4	Very watery, soft, spongy	Very small, flattened completely white, mostly seed coat.
5	Still soft, not as watery, inner pericarp fleshy-no cracks	Larger than 4 weeks, flat; white or may just turning pink at one end.
6	Inner pericarp tissue beginning to show cracks	Torpedo shaped; generally pink at embryonic-axis end of kernels.
7	Inner pericarp beginning "cottony" appearance	Torpedo to round shaped; embryonic axis end of kernel pink; other end white to light pink.
8	Inner pericarp beginning to dry out-cracks more numerous	Round, light pink all over.
9	Inner pericarp white but beginning to show brown splotches	Dark pink at embryonic axis end, light to dark pink elsewhere.
10	Many dark brown splotches on inner pericarp	Large, generally dark pink all over; seed coat beginning to dry out.
11	Inner pericarp almost completely brown	Dark pink, may show imprint of pericarp on seed coat in places; seed coat drying out.
12	Black splotches appearing on inner pericarp	Same as 11
13	Black splotches over at least 1/2 the pericarp	Seed coat beginning to turn brown
15	Black splotches throughout pericarp	Seed coat almost all brown, imprint of pericarp seen over large part of kernel.

Results and Discussion

FRUIT — PERICARP

The first stage in fruit development was taken as the time just after the peanut peg has turned horizontally and fruit enlargement was visible. The fruit was analyzed *in toto* until the seed coat and seed were large enough to be removed intact from the parenchymatous tissue of the inner pericarp (stage 4).

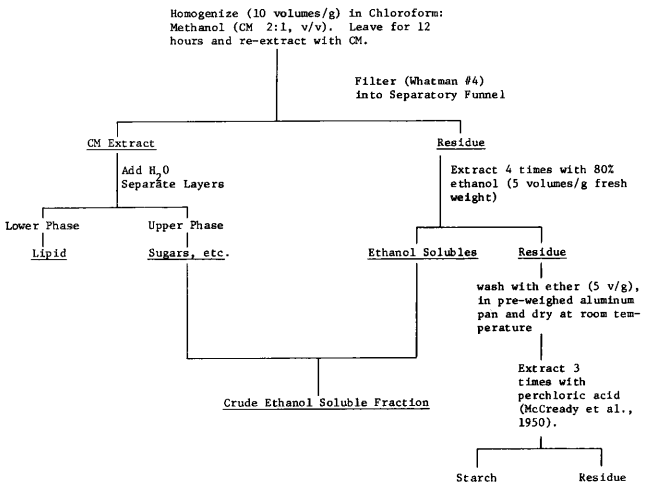

Fig 1. Diagram for obtaining the residue, starch, crude-ethanol-soluble, and lipid fractions from the peanut fruit parts.

Fresh weight of the fruit (Figure 2A) increased rapidly; maxima were reached at stage 7 for the first harvest date and at stage 5 for the second and third harvest dates. The differences in fresh weight between the first and later harvest dates were due almost entirely to moisture content (Figures 2B, C, D); other components did not differ markedly at comparable harvest dates. The low moisture content in fruits harvested on the second, third, and fourth harvest dates could have been caused by low soil moisture; in North Carolina moisture stress is common by early August (Sneed, 1971). The decrease in fresh weight beyond stage 6 or 7 suggested not only that soil moisture stress influenced the moisture in the fruit (Schenk, 1961) but that the metabolic role of the pericarp as a substrate reserve for the developing seed was diminishing and beyond stage 8 had practically ceased.

Increases in the extraction residue weight of the pericarp (Figure 2B) suggest that fibrous materials were deposited in the pericarp throughout maturation. This observation appears to conflict with Schenk's (1961) data showing that the dry weight of the pericarp remained static after the third week of development (our stage 6). Since he did not separate the pericarp into its parts (residue, starch, etc.), however, the dynamic changes occurring in the pericarp throughout maturation could have been masked.

The role of the pericarp as a storage organ for metabolic reserves which are used subsequently in seed development is evident from the changes in sugar content during fruit maturation. Sugar content was maximum, 37 to 47 mg, between stages 5 and 6, then declined rapidly, and reached a stable level of 5 to 7 mg per individual pericarp at stage 10 (Figure 2C). Possibly the decrease in sugar level contributed to the increasing residue weight of the pericarp through the conversion of sugar to hemicellulose. This conversion process in the

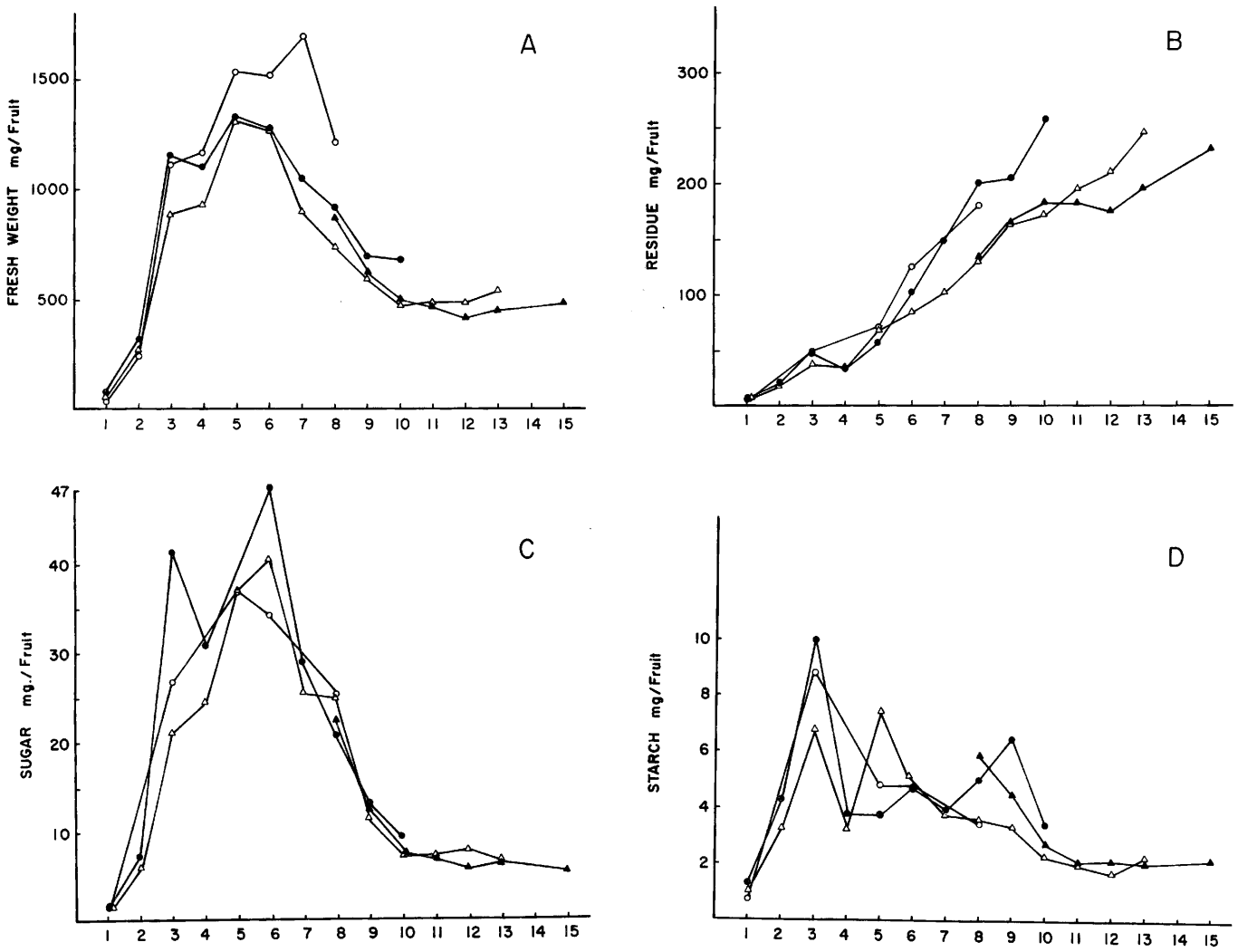

Fig. 2. Influence of developmental stage on (A) Fresh Weight: (B) Extraction Residue; (C) Sugar; and (D) Starch Content of peanut fruit: beyond stage 3 - pericarp at: First harvest (0 - 0); Second harvest (● - ●); Third harvest (△ - △); Fourth harvest (▲ - ▲).

pericarp could compete with the seed for the available photosynthate during the later stages of maturation.

A relationship between starch and sugar levels in the pericarp could be postulated since sucrose is a precursor for starch. However, starch accumulated early, reaching a maximum at stages 3 to 5, and then rapidly declined to a constant level at stage 10. Thus, these data indicate that starch was accumulated as a metabolic reserve only during very early stages of pericarp development.

SEED COAT

Studies on the seed coat commenced at stage 4. The initial stage combined the seed coat and seed since the interior cavity consisted of liquid endosperm, minute cotyledons and embryonic axis. Fresh weight of the seed coat (Figure 3A) increased rapidly to a maximum of 280 to 310 mg at stage 9 and then decreased to between 70 and 80 mg/seed coat. Residue weight (Figure 3B) was maximum at stage 9 for the third and fourth harvests and at stage 8 for the second harvest. Residue weight declined rapidly between stages 9 and 11; final weight being between 12 and 13 mg per seed coat.

Sugar content in the seed coat reached it maximum between stages 8 and 9, then rapidly declined until stage 11. The continuing decline suggests that the seed may be drawing metabolic reserves from the seed coat even though the seed were classified as over-mature (stage 15). Comparison of the maximum sugar level in the seed coat and pericarp (Figures 2C and 3C), 7-11 mg vs. 40-45 mg, respectively, indicates the greater sugar reserve potential of the pericarp. In contrast, approximately equal maximum amounts of starch accumulated in the seed coat and pericarp, stages 7 and 3, respectively (Figures 2D and 3D). Stages of maximum accumulation would be indicative of high photosynthate retention and low efflux to subsequent fruit parts. The onset of a rapid decline fol-

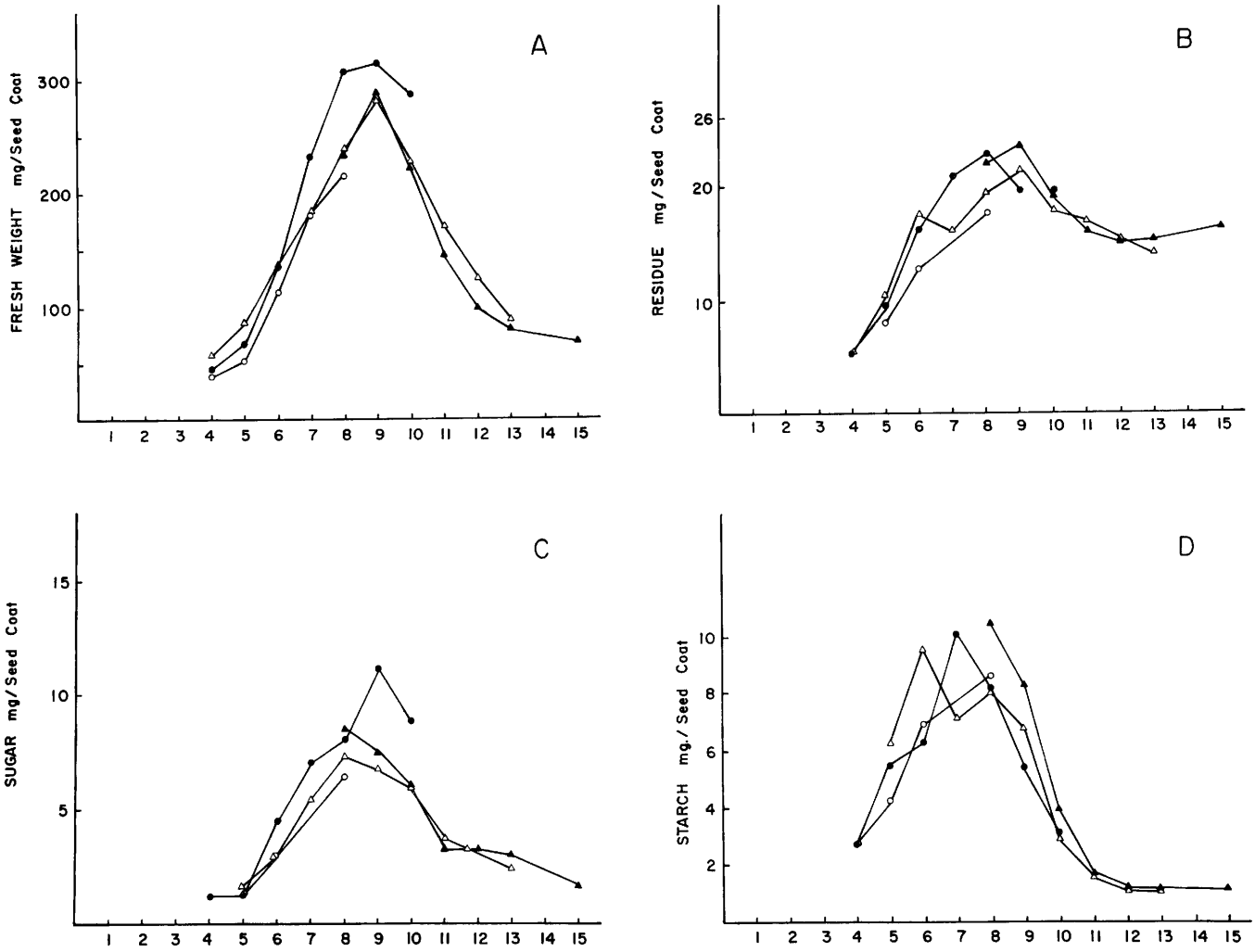

Fig. 3. Influence of developmental stage on (A) Fresh weight; (B) Extraction Residue (C) Sugar; and (D) Starch content of the peanut seed coat at: First harvest (0 - 0); Second harvest (● - ●); Third harvest (△ - △); Fourth harvest (▲ - ▲).

lowing the maximum levels could be used as an investigative marker indicating increased metabolic activity and development of the subsequent fruit part.

SEED

In peanut fruits, seed development does not become a dominant process nor are the seeds large enough for component analysis until midway through maturation. Therefore, analysis of seed components could not be undertaken until stage 5 when the seed first could be removed intact from the seed coat. At this stage the fresh weight ranged from 20 to 60 mg per seed and residue weight from 2 to 4 mg per seed. Fresh weight (Figure 4A) follows a typical sigmoidal growth pattern and reaches a maximum of 1.2 g at stage 13. Rates of increase in residue weight (Figure 4B) were essentially linear between stages 6 and 13 and the maximum residue weight was 340 mg; residue weight decreased in over-mature seeds, stage 15. From an economic standpoint peanut growers should consider the trend towards weight loss in over-mature seeds along with the necessity for maximum average maturity before harvest to maximize yield.

Sugars may be important in development of roasted flavor (Mason, *et al.*, 1969). For this reason and as biochemical precursors, a knowledge of changes in sugar content during maturation is important. Sugar content tended to increase throughout maturation (Figure 4C). In contrast, Mason, *et al.*, (1969) observed decreases in sucrose content of seed during the early stages of development and suggested that sucrose was not a good maturity indicator. However, their data are expressed on a μmole/gm fat free meal basis rather than a "per seed" basis. Pickett's (1950) values for total sugars, expressed on a per seed basis, are similar to our data for the third harvest date (Figure 4C). Peanuts at similar physiological stages vary in composition depending on the date of harvest. However, the general trend of our data suggests that total sugar content may serve as a maturity marker in large-seeded Virginia-type peanuts.

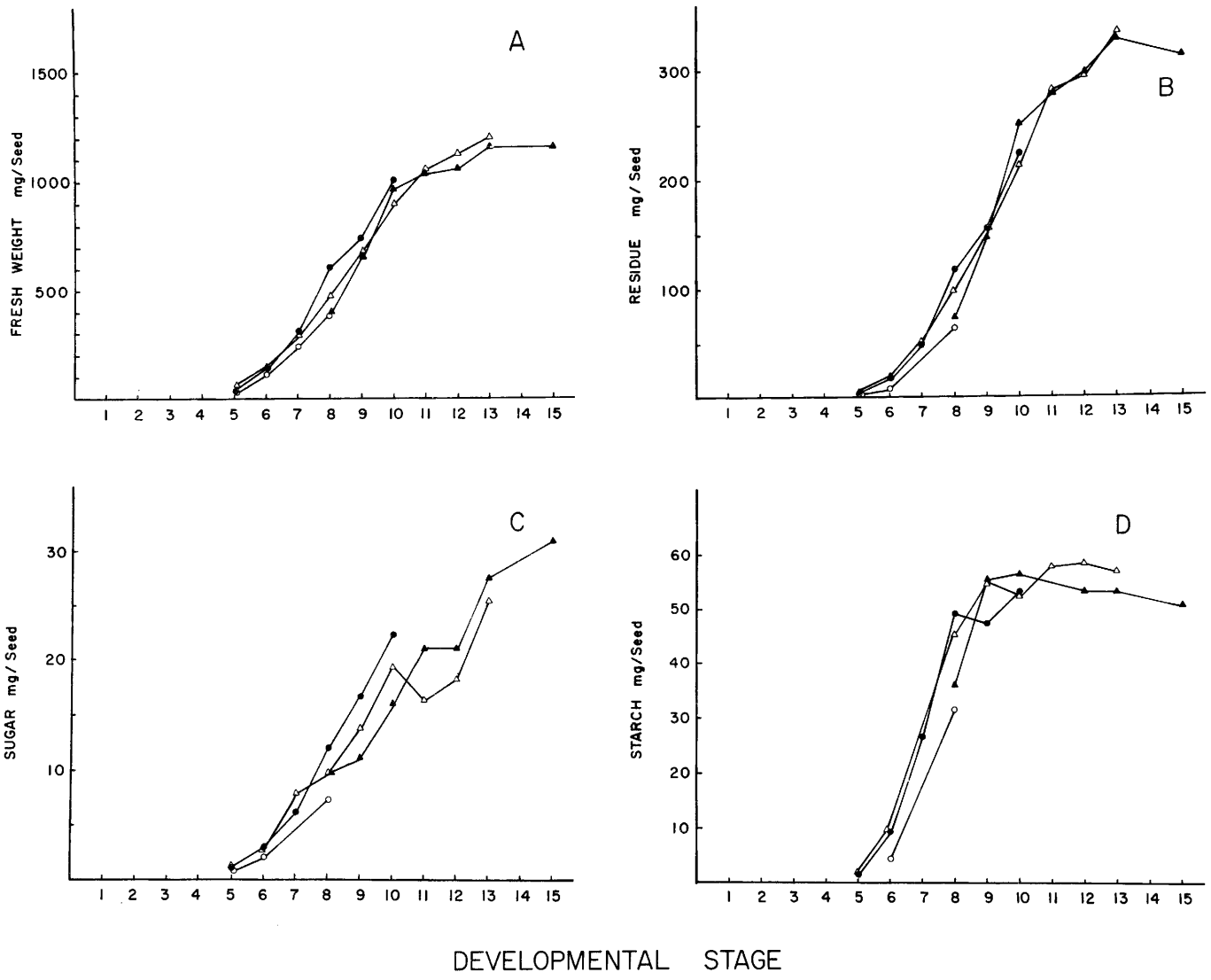

Fig. 4. Influence of developmental stage on (A) Fresh Weight; (B) Extraction Residue; (C) Sugar; and (D) Starch content of the peanut seed at: First harvest (0 - 0); Second harvest (● - ●); Third harvest (△ - △); Fourth harvest (▲ - ▲).

Starch is not normally considered a reserve resource in peanut seeds. However, in the early stages of maturity the seed may accumulate starch more rapidly than lipid, the final major reserve (Figure 4D). At maturity stage 8 starch and lipid content were approximately equal (Figure 5), 50-55 mg/seed. After this stage starch accumulation became minimal or ceased depending on the harvest period. A possible role of the starch reserves is to provide a sugar source during germination until the glyoxylate system is operating at a sufficient level to supply the germination energy requirements. This postulation is supported by the observations of Vyas, *et al.*, (1967) that starch reserves in peanuts are rapidly depleted during the first day of germination. Marcus and Velasco (1960) and Longo and Longo (1970) showed that the glyoxylate cycle requires a lag time before becoming fully functional. Gientka - Rychter and Cherry (1968) and Longo (1968) also showed that isocitritase arises by *de novo* synthesis during germination of peanuts.

The possible role of starch as a precursor of roasted flavor has not been studied to the knowledge of the authors. Investigations of its possible role might lead to an indicator of roasting flavor potential.

Lipid content was low initially and rate of accumulation became maximum between stages 7 and 11 (Fig. 5). The highest level was observed in the seed at stage 13. Beyond stage 13 the data suggest that lipid catabolism predominates lipid synthesis.

Schenk (1961) questioned previous work on lipid build-up in peanuts as to whether or not lipid synthesis was ever a minor part of the seed's metabolism during early maturation stages. Based on lipid percentage data, he concluded that lipid synthesis was a continuous process during the early stages of peanut seed development and that his results supported similar conclusions by Boufil (1951). Schenk (1961) also suggested that lipid synthesis was always the major anabolic process in the seed. Our studies do not fully support all of these conclusions. They do suggest that lipid syn-

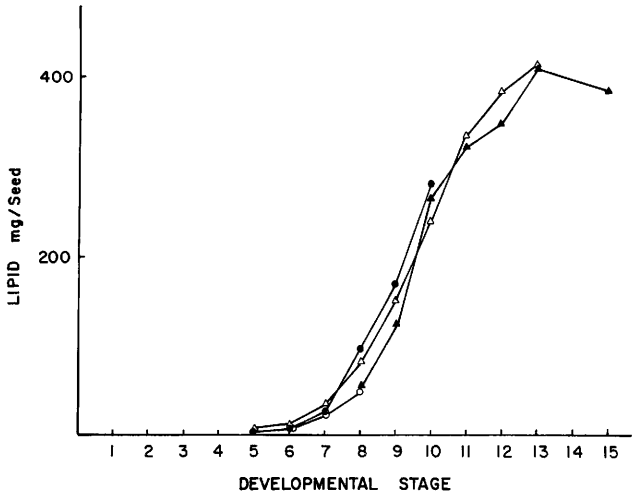

Fig. 5. Influence of developmental stage on peanut seed lipid content. First harvest (0 - 0); Second harvest (● - ●); Third harvest (△ - △); Fourth harvest (▲ - ▲).

thesis commences at a very early stage of seed development. Radioactive tracer studies by Pattee, *et al.,* (1974) also support this observation. However, the conclusion that lipid synthesis is always dominant to other storage processes is not supported. We have shown earlier that the starch content in stages 5 thru 7 is equal to or higher than the lipid content. Lipid represents 14 and 17 percent of the total components at stages 5 and 6, respectively. At stage 7 the lipid increased to 27 percent; at this stage starch content is also 27 percent. Beyond stage 7 lipid synthesis becomes the dominant reserve accumulation mechanism.

The differences between our conclusions and those of Schenk (1961) are due primarily to the manner of evaluating the peanut seeds as to their stage of development. Previous tests in our laboratory using peanut pegs tagged at the same zero time and harvested weekly, produced peanuts of quite divergent physiological maturity. Using our classification information the various physiological stages of development found on an individual plant can be more accurately defined. This is supported by the relative agreement in trend found between harvest dates for a comparable stage of development. This agreement also indicates that the selection of physiological stages within a harvest period was reproducible from one harvest to the next. This system of evaluating maturity has improved our ability to classify peanuts so that more definitive evaluations can be made on physiological and biochemical changes during development and maturation.

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