

# Changes in Carotenoid and Oil Content During Maturation of Peanut Seeds<sup>1</sup>

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## Abstract

To understand the changes in the color of peanut oil during maturation of the seeds, measurements were made of carotenoid and oil contents per kernel and carotenoid concentration of extracted peanut oil between the 4th and 12th weeks from pegging. Initially carotenoid concentration in the oil declined rapidly followed by a 50% decline between the 6th and 12th week. Changes in the carotenoid content and oil content of the peanut kernel indicated that the decrease in carotenoid concentration was due to a dilution produced by the rapid increase in oil content. Evidence is presented to indicate that the carotenoids are in areas separated from the oil containing spherosomes of the peanut kernel.

## Introduction

The loss of peanut oil color with maturity of the seeds is well documented. Holley and Young (1) showed the color reduction in peanut oil to be highly correlated with peanut maturity, but noted that an extraneous color loss occurred in all peanut varieties when slow cured. Spanish and Valencia types showed a higher rate of loss than Virginia and Bunch types. Emery et al. (2,3) suggested the use of peanut oil color as a maturity index and as a genetic marker of maturity inheritance. However, such an index of maturity is subject to variation due to loss of color during curing (3-5).

The pigments responsible for the oil color were postulated to be carotenes by Sharon (6) and xanthophylls by Kramer et al. (4). Pattee and Purcell (7) isolated and identified the carotenoid pigments of peanut oil and showed  $\beta$ -carotene and lutein to be the major carotenoid pigments present in oil from immature peanuts, and that the total carotenoid concentration in oil from fully matured peanuts was insignificant by comparison.

The purpose of this study was to investigate the changes in carotenoid concentration in peanut oil and total carotenoid content of the peanut kernel throughout maturation, and to determine the cause for the change in oil color which occurs in the oil during maturation of the seeds.

## Experimental Procedures

### Plant Material

Peanut maturation samples (Var. NC-2) were harvested in 1966 and 1968 using the branching diagram for the two main cotyledonary laterals as described by Gupton et al. (8). The average initial pegging date was determined by field sampling at weekly intervals until pegging was judged to have occurred at the desired positions on approximately 75% of the plants.

Peanut pods were harvested from positions

1,2,3,6,7,8 on the main cotyledonary lateral and the kernels were selected for uniformity of development and maturity.

Sampling commenced on the 4th week following pegging and continued at weekly intervals through the 12th week. In 1968 an additional sample was taken at 15 weeks. The samples were immediately cured in a forced-air dryer at 22 C to 8% moisture and stored at 5 C and 50% R.H. until analyzed.

Preliminary studies on sample size indicated that the smallest sample size allowable was 100 kernels or 50 g. Samples were taken in triplicate and weight and number of kernels were recorded.

### Extraction of Carotenoids and Oil

The samples were processed by the procedure outlined in Figure 1. The carotenoids and oil were extracted from peanut kernels by grinding in two volumes of methanol for 2 min using a blender. The slurry was mixed with Hyflo Supercel (2.5 g/50g peanut kernels), filtered with a Buchner funnel using filter paper coated with Hyflo Supercel and the filtrate saved. The filter cake was suspended in 2 vol of acetone-hexane (1:1) and refiltered. The residue was washed with 2 vol of acetone-hexane (1:1) and discarded. The combined acetone-hexane filtrates were then transferred to a separatory funnel where two distinct phases separated after standing. The bottom phase, consisting of acetone and water extracted from the tissue, was transferred to another separatory funnel and mixed with 1 vol of ethyl ether and 2 vol of water by gently swirling. After formation of distinct phases the aqueous phase was withdrawn and discarded. The ether phase was then washed twice

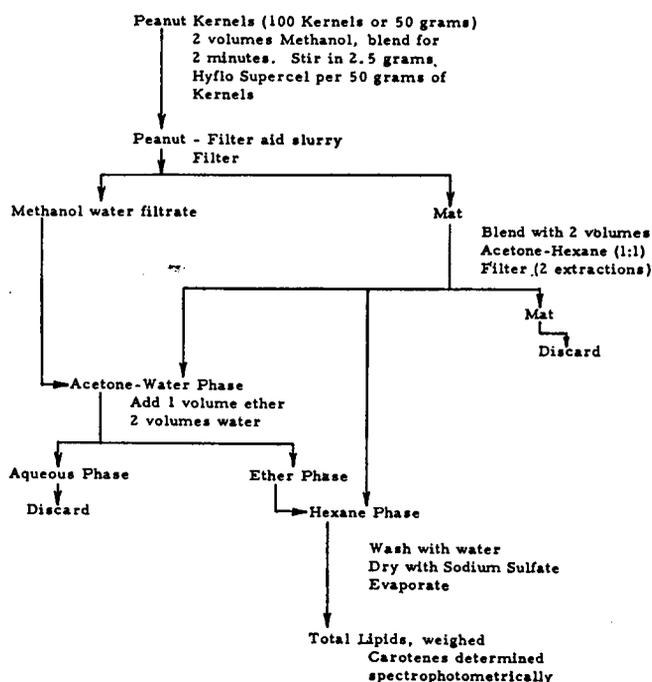


Fig. 1. Scheme for extracting carotenoids and lipids from peanut kernels.

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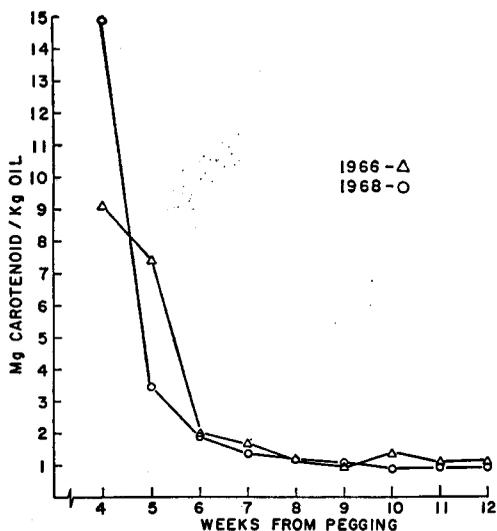


Fig. 2. Influence of maturity on the carotenoid concentration in solvent extracted peanut oil from cured peanuts.

with an equal volume of water. The original upper hexane phase was washed by swirling several times with 1 vol portions of water. The ether and hexane fractions were combined, dried with sodium sulfate and evaporated in vacuo to leave the oil residue containing the carotenoids. This residue was weighed to determine the per cent of oil and then made to 25 ml with hexane. Two g of sodium sulfate were added to remove any traces of water in the solution and the carotenoids were measured on a Cary Model 15 spectrophotometer at 450 nm. An extinction coefficient of 0.25 was used to determine their concentration in mg/liter.

Oil was also extracted from the peanut kernels by use of a Carver press and the carotenoid concentration in the oil determined spectrophotometrically after a clearing by centrifugation at  $40,000 \times g$  for 15 min.

### Results and Discussion

To understand the reduction of color in peanut oil during maturation changes of carotenoid concentration of peanut oil must be determined. Figure 2 presents data, collected in 1966 and 1968, which give the changes in carotenoid concentration in solvent-extracted peanut oil. The patterns of these changes were about the same for both years. In 1968 con-

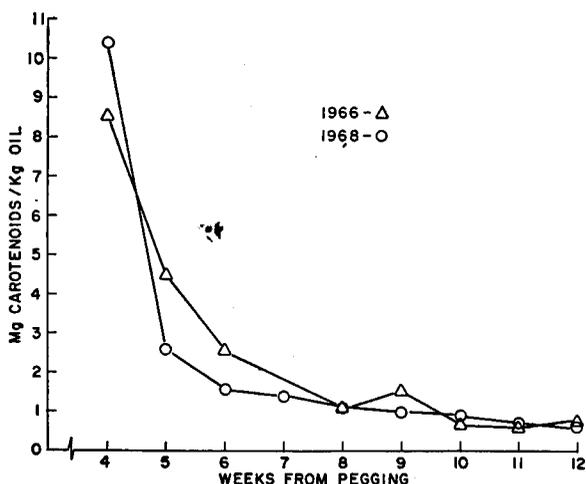


Fig. 3. Influence of maturity on the carotenoid concentration in pressed oil from cured peanuts.

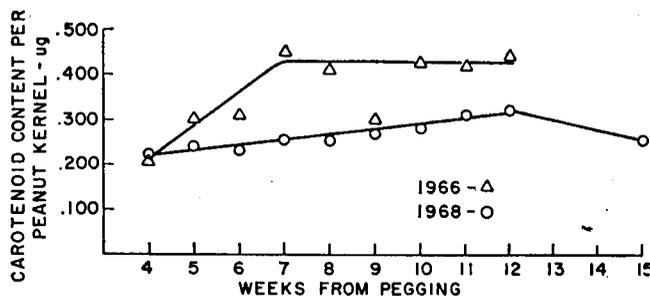


Fig. 4. Effect of maturity on the carotenoid content of peanut kernels.

centration at the initial harvest date was higher than in 1966 and reduction was more rapid up to the sixth week from pegging. Throughout the remainder of the maturation period the data generally agree and carotenoid concentration decreased 50% between the 6th and 12th weeks from pegging. Differences in environmental conditions due to cool weather which delayed pegging and water stress during kernel formation in 1968 are thought to be responsible for the differences in carotenoid concentration at the fourth and fifth weeks from pegging.

When oil is pressed from the peanut kernel, maturational changes of carotenoid concentration are similar (Fig. 3), but the values from pressure extraction are generally lower than those from solvent extraction. This difference indicates that the carotenoids in peanut kernels are in areas separated from the oil containing spherosomes. Attempts to localize the carotenoids in the cell have not revealed well defined carotenoid containing plastids. The distribution of carotenoids may be similar to that found in squash by Purcell et al. (9) who reported carotenoids to be distributed throughout the cytoplasm rather than in distinct plastids.

To ascertain if concentration of carotenoids in peanut oil was decreased by carotenoid destruction, the carotenoid content per peanut kernel was calculated (Fig. 4). The 1966 data indicate that carotenoid content increased rapidly between the fourth and seventh week from pegging and then maintained a nearly constant level. The 1968 data show a small but consistent increase throughout development and maturation. The lower levels of carotenoid content in 1968 are thought to result from the interaction of environment differences of the two years, as previously noted, and a carotenoid degradation process (10). This degradation process is not completely understood at the present time.

It is evident from the carotenoid per kernel data (Fig. 4) that the reductions in carotenoid concentration in the oil (Fig. 2,3) did not come about through carotenoid degradation, i.e. the amount of carotenoid per kernel did not decrease.

One of the most rapidly increasing fractions of the peanut kernel during development and maturation is the lipid fraction. Data from 1966 and 1968 indicate that the oil content of the kernel increases in a nearly linear manner up to the time the kernels are considered mature at 12 weeks after pegging (Fig. 5). In 1968 additional data, collected at 15 weeks after pegging, indicate that oil content decreases in over-mature peanuts. This loss might be due to the use of lipids as a substrate for respiration (11). Data, presented as per cent oil indicate that the oil fraction increased rapidly, in relation to other constituents during the fourth to seventh week from pegging but at subsequent harvest dates this increase

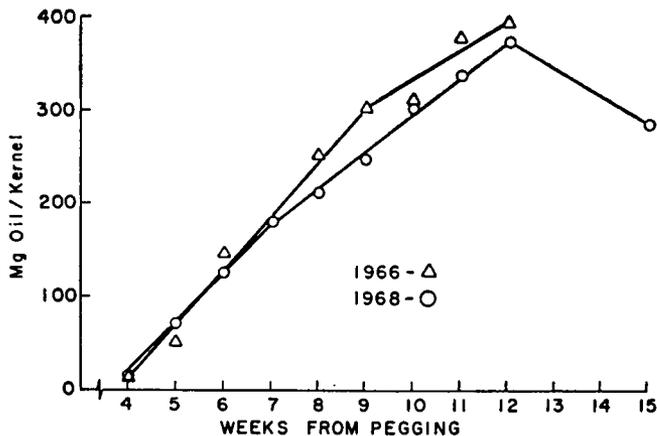


Fig. 5. Influence of maturity on the oil content of peanut kernels.

is not as great (Fig. 6). The before mentioned effect of environmental stress on the plant in 1968 also appears to have influenced the rate of oil synthesis in relation to other constituents, as indicated by the lower percentages when compared to the 1966 data.

Comparison of the increase in oil with the very slight increase in carotenoid content shows that the reduction in carotenoid concentration of extracted or pressed oil is due to a dilution effect by the oil. Perhaps when the peanut oil is extracted by pressing, as is done when using oil color as a maturity index or as a genetic marker of maturity inheritance, the large oil containing spherosomes are ruptured allowing oil to flow out. The resulting mass of oil could act as a solvent extracting the carotenoids from the pigment containing areas. The lighter color of the pressed oil might be due to less than quantitative leaching of carotenoids by the oil expressed from the spherosomes.

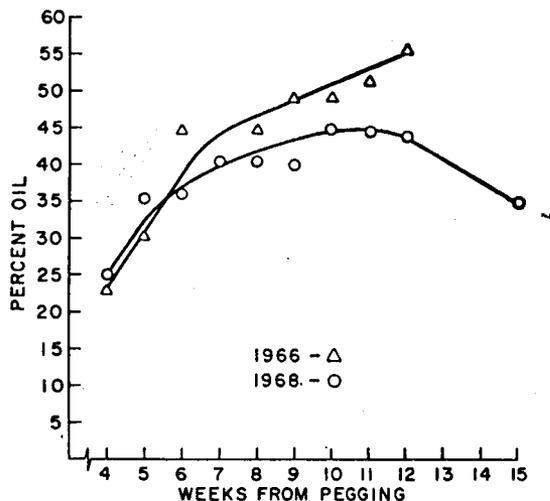


Fig. 6. Changes of per cent oil in peanut with maturity.

#### ACKNOWLEDGMENT

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