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Seed Composition and Physiological Changes in Thai Peanut cv. Kaset 1 and Tainan 9 during Maturation

Parichart Promchote[1,2], Juangjun Duangpatra[2*] and Wanchai Chanprasert[2]

ABSTRACT

Physiological changes and chemical compositions in peanut seed during maturation were investigated in two Thai cultivars: large-seeded Kaset 1 and medium-seeded Tainan 9. Nine maturity stages (5-13) of both cultivars designated by the Physiological Maturity Index (PMI) method were studied to determine its appropriateness for maturity classification. Physiological maturity (PM) of Kaset 1 was evident at stage 10 with a seed moisture content of 36.37% while in Tainan 9 PM was attained at stage 11 with a seed moisture content of 35.48%. Peanut seed size, seed weight and the seed/hull dry-weight ratio increased as the seed matured and reached a maximum at PM, while seed moisture content declined and was stable from PM onwards. In Kaset 1, seed dormancy was found at stage 5 and was maximized (90%) at stage 9, while in Tainan 9, seed dormancy was found marginally at stage 12 (4.0%) and 13 (1.3%). During seed maturation of both cultivars, oil accumulation increased rapidly while carbohydrate content declined. Protein content did not change notceably during seed development. The oleic acid and the O/L (oleic/linoleic acid) ratio also increased while palmitic, linoleic, ecosenoic and behenic acid contents decreased. At maturity (stage 13), oil, carbohydrate and protein contents in Kaset 1 seed were 55.9, 21.7 and 20.1%, respectively and in Tainan 9 seed were 54.8, 21.5 and 21.2%, respectively. Regardless of the difference in seed size, the oil content of Kaset 1 and Tainan 9 peanut seeds was marginally different, while the O/L ratio of Kaset 1 seed was higher than that of Tainan 9 seed. It can be concluded that the PMI method was appropriate for classifying the maturation of peanut fruit and predicting the harvest date for Thai peanut cultivars.

Key words: *Arachis hypogaea*, maturation, oil, protein, fatty acids, seed germination, viability, dormancy

INTRODUCTION

The physiological characteristics of seed and its chemical composition are factors related to seed maturation and affect both seed quality and the quality of any peanut products. Non-uniformity of fruit and seed maturation is also an important problem in peanut production. Several methods for determining fruit maturation have been reported (Johnson *et al*., 1976; Sanders *et al*., 1980; Pattee, *et al*., 1974a). The proper time to harvest was considered to be when the greatest weight of sound mature kernels was available, resulting in the highest yields and grades (Pattee *et al*., 1980; Sanders *et al*., 1980; Williams and Drexler, 1981). However, studies on the physiological changes of

1 Present address: Copenhagen University, Frederiksberg Campus, Copenhagen, Denmark.
2 Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: agrjua@ku.ac.th

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peanuts as they mature are complicated, because the maturation process is continuous and cannot be separated into distinct stages. Methods to determine the relative physiological maturity are all based on internal or external physical and morphological characteristics of the hull, seed coat, and seed. Pattee *et al.* (1974a) found that specific definite physical and morphological characteristics on a weekly basis described the physiological stages of maturity, rather than a weekly progression of development and called these characteristics the Physiological Maturity Index (PMI). PMI methods have been used to investigate physiological changes in volatiles and the lipoxigenase enzyme (Pattee *et al.*, 1970); carbohydrates (Pattee *et al.*, 1974a); ^{14}C distribution (Pattee *et al.*, 1974b); lipase (Sanders and Pattee, 1975); tannins (Sanders, 1977); lipid class composition (Sanders, 1980a); fatty acid composition (Sanders, 1980b); and triacyl-glycerol stereospecific structure (Sanders, 1979). The changes detected in each study indicated a logical physiological progression and verified the accuracy and reproducibility of the PMI (Sander *et al.*, 1982a).

The development and maturation of peanut seed including its size, weight, germination, dormancy, viability and storability can be investigated by following tagged flowers and periodically harvested pods (Saisawat, 1980). However, it is difficult to predict the harvest timing to receive good quality seed, therefore PMI should be used for considering the development and maturation of peanut seed, especially for peanut cultivars grown in tropical regions such as Thailand. PMI as a means of examining the fundamental chemical composition can also be useful in peanut harvesting. The objective of this study was to investigate the physiological and compositional changes at different stages during seed development and maturation. The physiological Maturity Index (PMI) method described by Pattee *et al.* (1974a) was evaluated its appropriateness in classifying peanut fruit maturity. Two Thai peanut cultivars, Kaset 1 (large-seeded cultivar) and Tainan 9 (medium-seeded cultivar) were selected to represent peanut cultivars grown in Thailand. The results of the study were considered to be advantageous in assisting the appropriate utilization of seed with a range of maturing times for both planting and product manufacturing.

MATERIALS AND METHODS

Plant material

Peanut cultivar Kaset 1, the promising large-seeded variety and Tainan 9, the local recommended medium-seeded cultivar were grown in July 1999 at the National Corn and Sorghum Research Center, Nakhon Ratchasima province, Thailand. A plant spacing of 50 × 20 cm with 2 seeds/hill was used in this study. Experimental plots were irrigated at 7-day intervals before pegs touched the soil surface and thereafter of 14-day intervals until harvest. Harvesting was done every seven days during 12 to 18 weeks after planting. Plants were uprooted, peanut pods were washed with tap water to remove soil, surface dried with a cloth, then packed in ice boxes and transported to the laboratory. Peanut fruits were opened and classified into nine maturity stages (5 to 13) depending on pod and seed morphological characters and the color of internal pericarp according to criteria modified by Pattee *et al.* (1974a) as shown in Table 1.

Oil, protein, carbohydrate and ash contents

Seed samples from different stages of maturity of each cultivar were examined for oil, protein, carbohydrate and ash content by proximate analysis (AOAC, 1990). Seeds were ground and the oil was extracted for 8 h with petroleum ether in a Soxhlet apparatus. The extracted oils were dried and the solvent removed at 80-90°C using a hot-air oven. Ash content was

Table 1 Physiological maturity index (PMI) by Pattee *et al*. (1974a) used to estimate kernel age.

Stage	Pericarp	Kernel
5	Still soft, not as a watery inner pericarp fleshy-no cracks	Flat; white or may just be 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	25-75% dark brown splotches on inner pericarp	Large, generally dark pink all over; seed coat beginning to dry out
11	Over 75% dark brown splotches on inner pericarp	Dark pink, may show imprint of pericarp on seed coat inplaces; seed coat drying out
12	Black splotches appearing on inner pericarp	Same as 11
13	Over 50% black splotches on inner pericarp	Seed coat beginning to turn brown

determined by incineration in a muffle furnace at 600°C for 3 h. The nitrogen content was estimated by the Kjeldahl method and then converted to a protein content using a conversion factor of 5.46. Carbohydrates were estimated from the difference between the total dry weight and the concentration of protein, oil and ash.

Fatty acid composition

Oil extraction was carried out according to the method of Bligh and Dyer (1959). Fatty acid methyl ester was prepared by transmethylation with 0.5 N methanolic sodium hydroxide and boron trifluoride reagent (AOAC, 1990). The fatty acid methylesters of the total lipids were analyzed by gas-liquid chromatography (GLC) using a capillary column (50 m × 0.22 mm). The column temperature was programmed from 66°C (3°C/min) and the injector temperature was 250°C. A standard fatty acid methyl ester mixture was run and the results used for identifying sample peaks. Fatty acid levels were estimated on the basis of peak areas of known concentration of the standards. Percentages were determined for: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), arachidic acid (20:0), eicosenoic acid (20:1), behenic acid (22:0), lignoceric acid (24:0) and the oleic/linoleic ratio (O/L ratio).

Seed size, seed dry weight, seed/hull dry weight ratio, seed moisture content

Twenty fresh pods were randomly selected and hand shelled. Twenty seeds per replication were sampled to measure length and width. Seed length was mesured as the maximum distance between the radicle and the chalazal ends, while seed width was mesured as the maximum diameter of the plane perpendicular to the interface of cotyledons.

Twenty fruits were used to determine fresh seed weight and fresh hull weight. Dry weight was also measured after drying in a hot-air oven at 105°C, for 24 h and the seed/hull dry weight ratio was calculated. Moisture content percentage was determined on a wet-weight basis. All measurements were replicated four times.

Seed viability, seed dormancy, seed germination, seedling dry weight

Fresh fruits of each maturity stage were hand shelled to obtain 100 seeds for a fresh-seed germination test. Other portions of seeds at different maturity stages were air-dried for 10 days at room temperature. They were then hand shelled and 100 air-dried seeds were selected to analyze seed germination, viability and dormancy. Four replications of 25 seeds were planted in a plastic box containing sand under room condition (25 to 30°C). Moisture content was maintained at field capacity throughout the germination period. Germination counts were done at five days and 10 days after planting, respectively. The germination percentage was calculated based on the number of normal seedlings. The number of firm-and-fresh-ungerminable seeds (considered as dormant seed) was counted and seed viability was calculated by combining the number of normal seedlings and dormant seeds.

RESULTS

Seed size, seed dry weight, seed/hull dry weight ratio, seed moisture content

The evaluation of the development and maturation stages of Kaset 1 and Tainan 9 peanut seeds showed that seed width and seed length, seed dry-weight and the seed/hull dry-weight ratio increased as the seed matured, while seed moisture content declined (Figures 1A and 1B). In the early stage (stage 5), seed dry-weight of Kaset 1 and Tainan 9 was lowest (0.008 and 0.005 g/seed, respectively), while seed moisture content was highest (85 and 83%, respectively). After stage 5, the seed dry weight of both cultivars increased rapidly. Kaset 1 peanut reached a maximum dry weight of 0.552 g/seed at stage 10 while Tainan 9 attained a maximum dry weight of 0.459 g/seed at stage 11. The seed/hull dry weight ratio was found to be highest at stage 11 in both cultivars. Seed moisture content of both cultivars decreased rapidly from stage 6 until stage 11 and was 36% at stage 11. During stages 11 to 13, seed size, seed dry-weight and seed moisture content changed slightly.

Seed viability, seed dormancy and seed germination

Kaset 1 seed was very dormant during maturation while Tainan 9 seed showed only marginal dormancy at stage 12. Seed germination of Kaset 1 peanuts was 38% at stage 7 and decreased to less than 10% beyond stage 9. Fresh-ungerminable seed (dormancy) was found to increase from 40% at stage 7 to 90% at stage 9. Dormancy in Tainan 9 seed was only 4.0% and

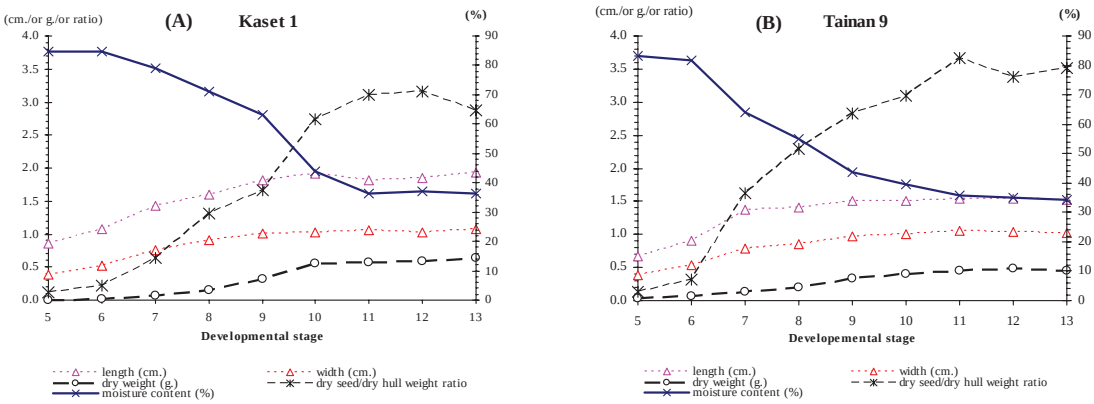

Figure 1 Seed size (length and width), dry weight, moisture content and seed/hull dry weight ratio of (A) Kaset 1 and (B) Tainan 9 peanut seed at different developmental stages.

1.3% at stage 12 and 13, respectively (Figure 2B). Seed viability of Kaset 1 peanut reached a maximum of 99% at stage 8 (before reaching PM) and remained higher than 90% thereafter, while in Tainan 9, seed viability was higher than 95% during stages 8 to 13 (Figures 2A and 2B).

Oil, protein, carbohydrate and ash

The chemical composition during seed maturation of Kaset 1 and Tainan 9 peanut seed are shown in Figures 3A and 3B. In Kaset 1 (Figure 3A), the major component of immature seed at stage 6 was carbohydrate (48%) and thereafter seed carbohydrate content decreased rapidly to approximately 22% at stage 11. Oil content was 24% at stage 6 and increased rapidly to its highest percentage at stage 13 (55.56%). Mature seed of Kaset 1 (stages 10 to 13) consisted of 52 to 56% oil and 20 to 23% carbohydrates. Protein and ash content throughout the developmental stages were not significantly different. In Tainan 9 (Figure 3B), changes in seed chemical composition were similar to those of Kaset 1. At maturity stage 7, oil and carbohydrate content were approximately equal at 38.2 and 35.6%, respectively. Thereafter, oil content increased rapidly and reached its maximum of 55.6% at stage 12. In contrast, carbohydrate decreased as seed matured to a

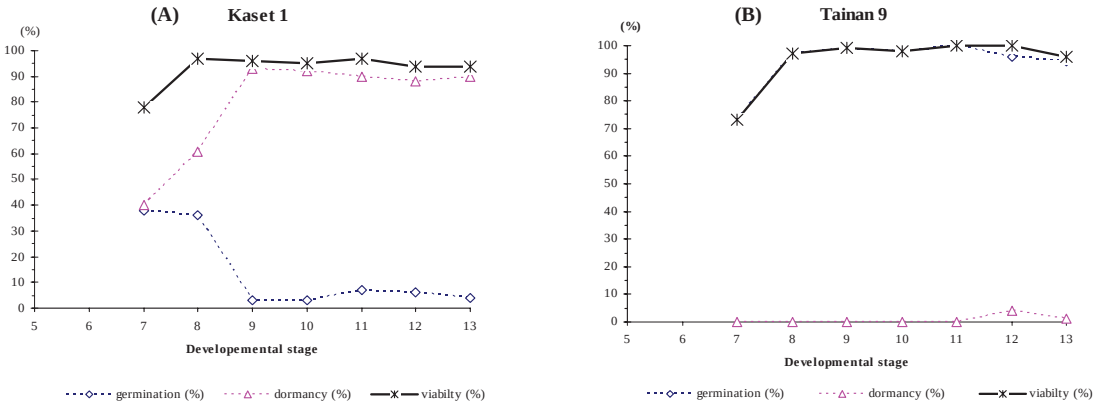

Figure 2 Germination, dormancy and viability of air-dried seed of (A) Kaset 1 and (B) Tainan 9 peanut at different developmental stages.

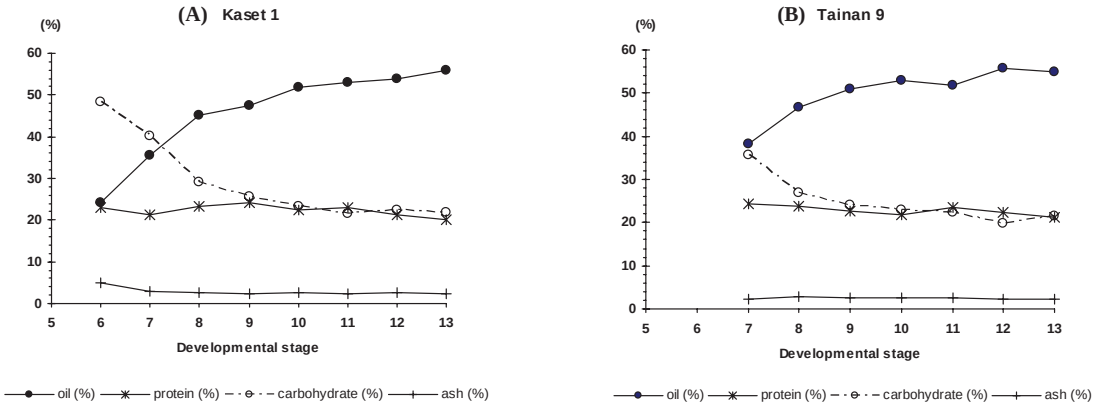

Figure 3 Oil, carbohydrate, protein and ash percentage of (A) Kaset 1 and (B) Tainan 9 peanut seed at different developmental stages.

minimum of 19.9% at stage 12. Protein and ash contents throughout the maturity stages changed marginally, ranging from 21.2 to 24.2% for protein and 2.3 to 2.8% for ash. At maturity (stage 13), the oil, carbohydrate and protein contents in Kaset 1 seed were 55.9, 21.7 and 20.1%, respectively and in Tainan 9 seed were 54.8, 21.5 and 21.2%, respectively.

Fatty acid composition

The fatty acid composition of Kaset 1 and Tainan 9 seed is shown in Tables 2 and 3, respectively. At each maturity stage, unsaturated fatty acid was found to be greater than 70% of the total fatty acids, with oleic and linoleic acids as the major acids. Different patterns of fatty acid changes during development were also found. Oleic acid increased as seed matured, while palmitic, linoleic, eicosenoic and behenic acids decreased. An increase of oleic acid and decreasing in linoleic acid resulted in an increase of the O/L ratio during seed maturation. The O/L ratio of Kaset 1 seed was 0.99 at stage 6 and then increased to about 2.0 to 2.2 in stages 9 to 13 (Table 2), while the O/L ratio of Tainan 9 seed increased from 0.94 at stage 7 to about 1.2 in stages 8 to 13 (Table 3).

DISCUSSION

The maturity stages of Kaset 1 and Tainan 9 peanuts were designated based on the physiological maturity index (PMI) described by Pattee *et al*. (1974a). It was found that seed dry

Table 2 Fatty acid composition of Kaset 1 peanut seed at different developmental stages.

Maturity stage	Fatty acid percentage								
	palmitic (16:0)	stearic (18:0)	oleic (18:1)	linoleic (18:2)	arachidic (20:0)	eicosenoic (20:1)	behenic (22:0)	lignoceric (24:0)	O/L ratio
6	15.34	2.18	35.89	36.00	1.33	1.92	6.49	0.16	0.99
7	13.33	3.21	44.32	28.96	1.83	1.58	5.49	0.43	1.53
8	11.52	2.85	49.40	27.87	1.71	1.47	4.38	0.31	1.77
9	13.44	3.36	51.35	25.05	1.53	1.22	3.07	0.35	2.05
10	11.39	2.82	54.94	24.94	1.39	1.20	3.12	0.14	2.20
11	11.32	3.01	52.74	25.07	1.09	1.20	3.23	0.24	2.10
12	11.25	3.69	52.96	24.86	1.71	1.19	3.33	0.34	2.13
13	11.06	2.83	52.42	25.95	1.72	1.07	3.71	0.44	2.02

Table 3 Fatty acid composition of Tainan 9 peanut seed at different developmental stages.

Maturity stage	Fatty acid percentage								
	palmitic (16:0)	stearic (18:0)	oleic (18:1)	linoleic (18:2)	arachidic (20:0)	eicosenoic (20:1)	behenic (22:0)	lignoceric (24:0)	O/L ratio
7	14.85	4.62	34.35	36.56	2.21	1.18	5.73	0.31	0.94
8	16.49	5.60	40.01	32.98	1.97	0.75	1.75	0.45	1.21
9	15.58	4.74	41.23	33.17	1.72	0.76	2.54	0.27	1.24
10	14.30	4.81	40.69	33.59	1.86	0.84	3.36	0.47	1.21
11	14.13	4.53	41.17	34.18	1.76	0.83	3.10	0.22	1.20
12	13.87	4.54	41.10	34.21	1.79	0.79	3.25	0.37	1.20
13	13.61	4.55	41.02	34.23	1.82	0.74	3.39	0.52	1.19

weight, moisture content and the seed/hull dry-weight ratio of both cultivars changed rapidly from stage 5 to 9. Maximum seed dry-weight for Kaset 1 and Tainan 9 were recorded at stages 10 and 11, respectively. Maximum seed dry-weight has been reported as a good index of physiological maturity (PM) for peanut seed (Saisawat, 1980) and also for other field-crop seeds (Delouche, 1976). The internal pericarp was white during stage 5 to 9 and changed to brown and black during stage 10 to 13. At stage 10 for Kaset 1 (PM), dark brown splotches were found on about 75% of the inner pericarp area. Maximum seed size and the seed/hull dry-weight ratio were also reached at this stage (Figure 1A). Moisture content decreased to its minimum (36.4%) seed dry-weight reached its maximum. In Tainan 9, dark brown splotches were observed on the entire inner pericarp area at stage 11 (PM) and similar to Kaset 1, the maximum seed/hull dry-weight ratio was also recorded at this stage when seed moisture content was minimal (35.8%).

Maximum viability and germination of Kaset 1 and Tainan 9 peanut seed were recorded at stage 8 before PM (Figure 2A and 2B). These results are similar to those reported by Saisawat (1980). Seed dormancy was prominent in Kaset 1, but negligible in Tainan 9. During stages 9 to13 of Kaset 1 maturation, dormant seed percentages exceeded 90%. In contrast, only 4.0% and 1.3% of dormant seed were recorded at stage 12 and 13, respectively. Sanders *et al.* (1982a) reported that mature seed of dormant peanut genotypes had relatively high content of an ABA-like inhibitor and phenolic compounds and low content of cytokinin and gibberellin. However dormancy was another factor influencing seed quality, especially maintaining high viability and vigor after PM and after harvest. Although Tainan 9 peanuts had a very low degree of dormancy, it has been reported as maintaining high germination, vigor and field emergence during storage when compared to some other cultivars (Phyo *et al.*, 2004).

Accumulation of oil content increased depending on seed maturation and reached its maximum at stage 13, which was in accordance with the results reported by Pattee *et al.* (1974a). Sanders *et al.* (1982b) studied oil characteristics in different maturity stages classified by the color and structural characteristics of the pod mesocarp (presented as the pod maturity profile, PMP) and reported that oil content increased significantly as seed matured and reached its maximum at the stage when the mesocarp color was orange to brownish-orange. This stage corresponded closest to stages 9 to 10 as classified by the PMI of Pattee *et al.* (1974a). Oil content decreased when the mesocarp color was black corresponding to PMI stages 11 to 13. In this study, although maximum oil content did not occur at the PM stage, the most rapid changes in total oil occurred in the early maturity stages and corresponded to the time of a rapid increase in seed dry-weight.

The carbohydrate changes of Kaset 1 and Tainan 9 seed were in contrast to oil accumulation. In the early maturity stage, seed contained a higher proportion of carbohydrate until stage 7 where the carbohydrate and oil concentrations were approximately equal. After stage 7, the carbohydrate content decreased at approximately the same rate as the increment in oil content. Pattee *et al.* (1974a) also reported that when the concentration of two substrates was approximately equal, starch accumulation became minimal or ceased. An increase in the lipids in the semi-mature seed caused a reduction in the carbohydrate content, because sucrose was considered to serve as a carbon source for the synthesis of both lipids and carbohydrates, therefore partitioning of carbon to these compounds must have involved direct competition for the products of sucrose metabolism. It is possible that some storage starch could be metabolized to hexoses which were then utilized in the synthesis of fatty acids (Murphy *et al.*, 1993). However, the results of this study supported similar conclusions by Pattee *et al.*

(1974a) that in the intermediate to mature-stages, lipid synthesis is always dominant to other storage processes. As the two Thai cultivars differed in average seed size, mature seed at stage 13 contained nearly the same amount of oil and carbohydrate percentages (approximately 55 to 56% of oil and 21 to 22% of carbohydrate in both cultivars).

The protein content in Kaset 1 and Tainan 9 peanuts did not change during seed development and maturation. In contrast, Basha *et al*. (1980) reported that the protein content of peanut seed increased on seed maturation. They compared high- and low-protein cultivars and found that from the immature to intermediate stages, high-protein cultivars deposited protein at a more rapid rate than low-protein cultivars. However, in the study of Basha *et al*. (1980) high-protein cultivars contained 40% protein content, while Kaset 1 and Tainan 9 seed contained less than 25% protein content. This may be the reason why protein content increased during maturation in the study of Basha *et al*. (1980).

Fatty acid composition is one of indicators of lipid quality. The dominant fatty acids of Kaset 1 and Tainan 9 peanut seed were oleic, linoleic and palmitic acid. Oleic acid increased upon seed maturation while palmitic, linoleic, eicosenoic and behenic acids decreased. This tendency was in accordance with the results reported by Sanders *et al*. (1982b). In Tainan 9 peanut seed, arachidic acid tended to decrease during maturation. This result was the same as that reported by Young *et al*. (1972) and Sanders *et al*. (1982b), but the level of arachidic acid in Kaset 1 seed did not change during maturation. Stearic and lignoceric acid levels in Kaset 1 and Tainan 9 seed changed during maturation which was contradictory to the results reported by Young *et al*. (1972) that mature seed contained high stearic and low lignoceric acid content but palmitic acid levels were stable in all maturity stages. However, previous reports demonstrated that the fatty acid profile in peanut seed was influenced by cultivars (Branch *et al*., 1990: Grosso and Guzman, 1995), production area and field environment-temperature, (Holaday and Pearson, 1974), harvesting date and maturity classification methods (Sanders and Bett, 1995).

Oleic acid and linoleic acid changed as seed matured, resulting in the enhancement of the O/L ratio with seed maturation. Oleic acid increased during the early stages, while linoleic acid decreased. Anderson *et al*. (1998) noted that oleic acid and linoleic acids were highly correlated ($r = -0.99$). Linoleic acid was formed by the desaturation of oleic acid stimulated by desaturated enzymes (Murphy *et al*., 1993). Peanut oil and peanut products that have a high O/L ratio are high in quality, have a high oxidative stability and a long shelf life (Sanders *et al*., 1982b; Branch *et al*., 1990; O'Keefe *et al*., 1993). Mature seeds of Kaset 1 and Tainan 9 peanuts have a different size (large and medium, respectively) but both were high in oil content 50 to 55% depending on management and environmental factors). With respect to the O/L ratio, oil quality of Kaset 1 was higher than that of Tainan 9. Mature Kaset 1 seed contained an O/L ratio of 2.0 to 2.2, while Tainan 9 peanut seed had a ratio of 1.2.

CONCLUSIONS

PMI, as described by Pattee *et al*. (1974a), is a good method for classifying the maturation of peanut fruit and predicting the harvest date for Thai peanut cultivars. The appropriate harvest time was found to be during stages 10 to 13 for the Kaset 1 cultivar and stages 11 to 13 for the Tainan 9 cultures, when peanut seeds showed a high seed viability and germination rate and a high seed/hull dry-weight ratio. Kaset 1 seed was very dormant during late maturation, while Tainan 9 seed was not dormant. Before PM, oil content rapidly increased but carbohydrate content decreased. At maturity, the oil and

carbohydrate content of both cultivars were more or less the same regardless of the differences in seed size. Fatty acid compositions also changed during peanut seed maturation and the amounts of oleic and linoleic acid as well as the O/L ratio were different between these 2 cultivars. Kaset 1 seed contained more oleic acid and a higher O/L ratio when compared to Tainan 9 seed.

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