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The Fatty Acid Composition and Cyclopropene Fatty Acid Content of the Maturing Okra (*Hibiscus esculentus* L.) Fruits

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Key words: Cyclopropene fatty acid; fatty acid; gas-liquid chromatography; *Hibiscus esculentus;* **lipids; okra.**

RINGKASAN

*Komposisi Asid Lemak dan Asid Lemak Cyclopropene bagi buah kacang bendi (*Hibiscus esculentus, *L.) yang sedang matang. Kacang bendi (*Hibiscus esculentus, *L.) dimakan manusia di seluruh dunia. Komposisi asid lemak buah kacang bendi yang di pungut pada hari ke 5, ke 6, ke 8 dan ke 12 selepas berbunga telah ditentukan oleh kromatografi cecair gas. Semakin buah-buah itu menjadi matang, semakin meningkat kandungan asid lemak C16 :0 dan semakin berkurangan kandungan asid lemak C18 :3. Lipid-lipid dalam buah belum matang mengandungi asid lemak C24 :0, manakala minyak yang didapati dari biji-biji yang matang sepenuhnya mengandungi asid lemak C21 :5. Asid lemak cyclopropene tidak dikesan dalam lipid-lipid buah yang belum matamg; sedikit sahaja asid ini di jumpai dalam minyak dari biji-biji yang matang sepenuhnya*

SUMMARY

*Okra (*Hibiscus esculentus, *L.) fruits are consumed as vegetable throughout the world. The fatty acid composition of the fruits harvested on the 5th, 6th, 8th and 12th day after flowering was determined by gas liquid chromatography. As the fruits matured, C16 :0 fatty acid content increased with a corresponding sharp decrease in C18 :3 fatty acid concentration. The lipids in immature fruits contained C24 :0 fatty acid, whereas the oil recovered from fully matured seeds had C21 :5 fatty acid instead. Cyclopropene fatty acids were not detected in the lipids of immature fruits, occurring only in small amounts in the oil of fully matured seeds.*

INTRODUCTION

Vegetables are appreciated for their freshness in addition to being a good source of vitamins and minerals. The seeded vegetables such as those from the family *Cucurbitaceae* and *Leguminoseae* also provide a reasonable amount of protein and essential fatty acids. Okra (*Hibiscus esculentus,* L.) also known as lady's finger or 'bendi', bears pod-like seeded fruits. It is native to Africa and is now widely cultivated throughout the tropics and sub-tropics largely for its fruits as vegetable. The green tender pods are consumed in a variety of ways, raw or cooked, sometimes canned or dried for later use. Being mucilaginous, they are often used in tropical cookery to thicken soups and curries. The eating quality and chemical composition of maturing okra have been examined by several workers (Sistrunk *et al.,* 1960 and Singh *et al.,* 1974). Okra fruits harvested on the 6th and 8th day after flowering were reported to possess optimum quality. The mature seeds were found to contain about 20% protein and 21% fat (Karakoltsidis and Constantinides, 1975).

Since okra belongs to the family *Malvaceae,* the oil in its seeds may contain cyclopropene fatty acids (Phelps *et al.,* 1965). Furthermore, it has been shown that as the seeds of the family *Malvaceae* mature, the proportion of cyclopropene fatty acids (CPFA) in their oils decreases, the highest amount occurring in the immature seeds (Yano *et al.,* 1972). The immature seeds in tender okra pods may, therefore, contain a higher proportion of CPFA in their oil when compared to matured seeds. The adverse effects of these fatty acids in experimental animals have been well documented (Mattson, 1973; Sinnhuber *et al.,* 1976; Ferguson *et al.,* 1976).

Key to author's name: Berry, S. K.

This study was undertaken to examine the fatty acids composition of the okra fruits at various stages of maturity in order to monitor the CPFA content.

MATERIALS AND METHODS

Materials

The okra fruits were procured from the Universiti Pertanian Malaysia farm. Methyl fatty acid ester standards were obtained through Sigma Chemical Company, St. Louis, MO, USA. The sodium methoxide reagent (0.5M) used for esterification of the lipids, was purchased from Supelco Inc., Bellefonte, PA, USA. All other reagents used for analysis were of analytical grade.

Planting and Harvesting of Okra:

Okra were planted during the month of October, 1979 at the research farm unit in four beds, each bed planted at an interval of one week. During the flowering period, the flowers were tagged bearing the date each morning. Okra fruits were harvested accordingly on the 5th, 6th, 8th and 12th day after flowering. Fully matured pods were harvested in February, 1980 to recover mature seeds.

Preparation of Fruits for Analysis:

The freshly harvested fruits were washed under the running tap water to remove any residual pesticides, dust, etc. The excess water on each fruit was blotted off. The fruits were then cut off their stalk and incised with a stainless steel spatula to remove the seeds. The empty pods (EP) and seeds (S) were weighed separately to calculate their ratio.

Extraction of Lipids:

A 100 g. each of EP and S was extracted separately with isopropanol followed by a mixture of chloroform : methanol (2:1, v/v) as described by Yano *et al.* (1972). The combined extract was treated with 0.2 volumes of 0.7% aqueous KCl to precipitate out the non-lipid matter and then washed with distilled water (Folch *et al.*, 1957). The purified extract was dried over anhydrous Na_2SO_4 and filtered and the solvents removed in a rotary evaporator under reduced pressure at 45°C to recover the lipids.

The Halphen colour test to ascertain the occurrence of CPFA in the seed oil and the methods to prepare methyl fatty acid esters and silver nitrate derivatives of CPFA were carried out as described previously (Berry, 1980).

Gas-Liquid Chromatography (GLC):

The methyl fatty acid esters and the silver nitrate derivatives of CPFA were analysed in duplicate on a Pye Unicam, series 204, gas chromatograph operated under the following conditions:

Columns: Column A (1.5m × 4mm I.D.) containing 10% diethylene glycol succinate adsorbed on 100-120 mesh Diatomite CAW was heated at 190°C with a carrier gas nitrogen (OFN) at a flow rate of 40ml/min. Column B (1.5m × 4mm I.D.) which was packed with 10% SE30 supported on Diatomite CAW DMCS, was heated at 220°C with a carrier gas nitrogen (CFN) at a flow rate of 50 ml/min.

Detector: The flame ionization detector was heated at 200°C. Hydrogen and air flow rate to the detector were maintained respectively at 10% in excess and 10 times of the carrier gas flow rate in the respective columns.

Injector: Temperature 200°C.

Gas chromatograph peaks were identified by comparison with pure methyl fatty acid esters through retention time relative to methyl octadecanoate on the two columns described above. The log of relative retention time on column A of the homologous series of saturated and unsaturated fatty acid methyl esters was plotted against the carbon number of the respective fatty acid ester to extrapolate the identification of fatty acids whose reference standards were not available. These were treated as tentatively identified. The identification of CFPA was carried out using *Sterculia foetida* (L). seed oil as reference. The latter is well known to contain both sterculic and malvalic acids (Phelps *et al.*, 1965).

The area percent of each sample fatty acid ester peak was obtained on Hewlett-Packard 3380A integrator in tandem with the gas chromatograph. A typical chromatogram is shown in Figure 1.

RESULTS AND DISCUSSION

The okra fruit, which is a pod, contains a number of seeds. The ratio between the empty pods and seeds at different stages of maturity was found to be between 1:6 to 1:3, decreasing

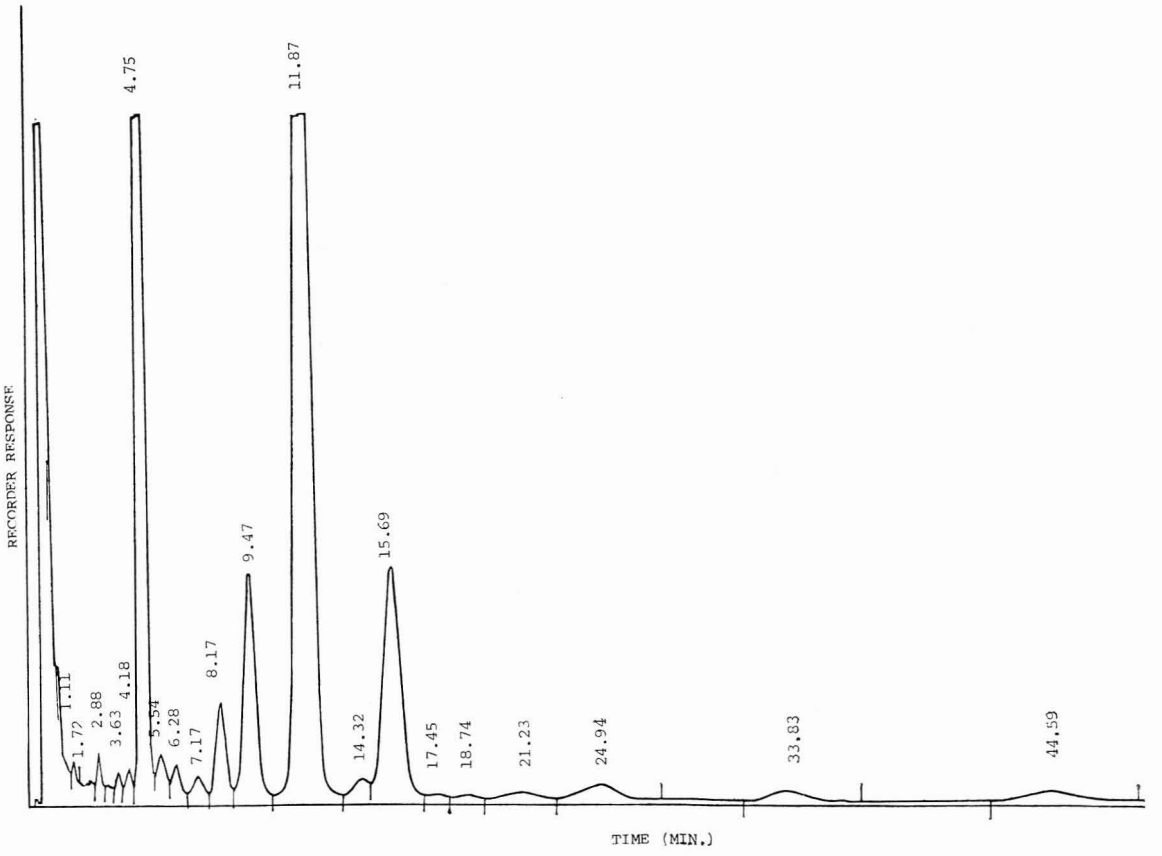

Fig. 1. GLC separation of methyl fatty acid esters of okra seed oil (12th day) on Diethylene glycol succinate column. Sample – 1 μl (5% in petroleum ether).

with maturity. The pods become fibrous and accumulate relatively less moisture whereas the seeds in the pods become larger in size as they mature. Both empty pods and seeds were analysed for their lipid content and fatty acid composition which is presented in Table 1.

The lipid content of EP and S harvested at different stages of maturity ranged between 1–9% and 3–20% (oven dried basis) respectively. The lipid content of EP decreased continuously between the 5th and 12th day of harvest after flowering. The lipid content of seeds, on the other hand, decreased until the 8th day of harvest after flowering and from then onwards it increased, the mature seeds containing the highest amount (*ca* 20%). The immature seeds tend to accumulate more of polar lipids; as they mature the non-polar lipids especially the glyceride content increases (Hitchcock and Nichols, 1971). In okra seeds, the oil synthesis was more rapid probably after the 12th day of flowering. The okra fruits were not analysed for lipid content and fatty acid composition between the 12th and the last day of their maturation as they become fibrous and inedible during this period of their growth.

The ratio between saturated and unsaturated fatty acids in the oils of both seeds and empty pods was found to be *ca* 1:3 with the exception of empty pods of the 12th day which had a ratio of *ca* 1:1. The oil recovered from the fully matured seeds, on the other hand, contained these fatty acids in the ratio of *ca* 1:2. The relative proportion of palmitic acid in seeds as they matured increased gradually with a corresponding sharp fall in linolenic acid content. The oil in seeds and empty pods harvested between the 5th and 12th day after flowering was found to contain lignoceric acid (C24:0) whereas the matured seeds contained C21:5 fatty acid instead. The fatty acids C21:4 and C24:4 were not detected in the oil of seeds as

reported by Karakoltsidis and Constandinides (1975), suggesting the probable influence of climatic and varietal factors.

Occurrence of CPFA :

The lipids in EP and S at different stages of their growth did not respond to the Halphen colour test indicating the absence of CPFA. The oil in fully matured seeds, however, produced a light pink colour, a positive Halphen test. The gas chromatographic analysis of the $AgNO_3$ derivatized methyl fatty acid esters of this oil did not show significant peaks due to CPFA derivatives when compared with the chromatogram of silver nitrate derivatized methyl fatty acid esters of *S. foetida* seed oil. From these observations it can be concluded that CPFA occur in okra seed oil only in small amounts which escape integration during GLC analysis.

TABLE 1

Lipid Content and Fatty Acid Composition Data of Okra.

Property	Day Harvested After Flowering								Fully matured and dried seeds
	5		6		8		12		
	EP	S	EP	S	EP	S	EP	S	
Lipids (%)*	9.60	10.30	9.60	9.20	1.90	3.30	1.1	4.1	19.87
Fatty Acid Composition (Area Percent):									
C 12:0	–	1.71	0.13	0.90	–	–	0.09	–	–
C 14:0	0.20	0.24	0.24	0.61	0.28	0.25	0.30	0.23	0.32
*t*C 14:1	← Trace →								
*t*C 15:0	0.07	0.11	0.06	0.12	0.04	0.13	0.16	0.11	0.02
*t*C 15:1	0.15	0.08	0.15	0.06	0.10	0.06	0.26	0.06	–
C 16:0	25.57	19.59	33.61	21.95	24.82	22.43	35.88	24.43	29.58
C 16:1	0.60	0.61	0.54	0.73	0.66	0.80	0.79	0.94	0.45
C 17:0	0.32	0.76	0.27	0.61	0.26	0.67	0.54	0.45	0.13
*t*C 17:1	0.59	1.21	0.37	1.08	0.38	1.23	0.36	0.60	0.25
C 18:0	1.27	2.49	1.55	2.05	1.10	1.77	2.08	2.53	3.80
C 18:1	6.11	12.92	7.59	12.61	7.06	11.62	6.43	18.12	19.72
C 18:2	45.47	45.72	40.63	47.10	43.01	49.13	38.75	45.19	44.21
C 18:3	17.38	13.79	12.34	9.97	20.89	9.68	9.65	5.67	0.28
C 19:0	← Trace →								
*t*C 19:1	← Trace →								
C 20:0	0.40	0.56	0.44	0.49	–	0.57	–	–	0.52
*t*C 20:2	0.65	0.14	0.41	0.20	0.62	0.20	0.46	0.08	–
C 22:0	0.64	0.72	0.83	0.50	0.45	0.63	0.36	0.59	0.22
Unknown	0.10	0.14	0.21	0.06	0.10	0.14	0.88	0.16	–
C 24:0	0.52	0.74	0.50	0.67	0.45	0.71	1.06	0.25	–
*t*C 21:5	–	–	–	–	–	–	–	–	0.60
CPFA	–	–	–	–	–	–	–	–	<0.10

* oven dried basis
t Tentative identification
EP = Empty pods; S = seeds

The absence of CPFA in immature okra seeds and their presence in mature seeds, though in small amounts, is contrary to the observations of Yano *et al.* (1972). Since plants show irregularity in the synthesis of lipids in their seeds (Hitchcock and Nichols, 1971), it is likely that CPFA develop in okra seeds after the 12th day of flowering but their concentration falls as they approach full maturity.

CONCLUSION

Okra should be consumed when tender to derive the maximum benefits of its freshness and some of the essential nutrients such as vitamins, minerals and polyunsaturated fatty acids. This study revealed that the okra of the 6th and 8th day after flowering were of the best quality. After this period, as they matured, they became more fibrous and hence inedible. Moreover, the matured okra tended to accumulate small amounts of CPFA in their seeds. These fatty acids could prove hazardous to man as they have been reported to cause cancer in rainbow trout, atherosclerosis in rabbit, death of rats fed with oil containing CPFA and numerous other physiological abnormalities in farm animals.

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