the two non-conjugated oils and also between the conjugated oils.

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Studies on Some Seed Fats of Cucurbitaceae Family

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A STUDY of the fixed oils obtained from the seeds of the plant family *Cucurbitaceae* is of interest because in some genera the component fatty acid composition is more or less simple while in others diene, triene, and tetraene acids have been found (1). The trends are similar in the seed fats of the allied families, *Rosaceae* and *Euphorbiaceae.*

In the present investigation the recent spectrophotometric technique introduced by Hilditch *et al.* (2) coupled with the methods of low temperature fractionation of fatty acids from organic solvents have been employed. Therefore the compositions of the seed fats reported are expected to be more accurate than those published previously (5-12).

In this part of the present series of investigations the seed fat composition of a) *Cucurbita Maxima* (Hubbard squash or gourd), b) *Lagenaria Vulgaris* (bottle gourd), c) *Citrullus Vulgaris* (watermelon), and d) *Benincasa Cerifera* (pumpkin) were investigated. The composition of these have been previously reported in the literature (5-12).

Experimental

The seeds were obtained from crops grown in the state of West Bengal, India. In each case the oil was extracted from ripe and dried seeds by petroleum ether (B.P. 40°-60°C.). The characteristics of the oils and seeds are presented in Table I. The contents of unsaponifiables were determined according to the methods recommended by the American Oil Chemists' Society (3). The mixed fatty acids of the oils were obtained by saponifying the latter with alcoholic caustic potash solution and then evaporating off the alcohol, extracting the unsaponifiable matters with sulphuric ether, subsequently decomposing the solution of the soap in water by HCl, extracting the liberated fatty acids with sulphuric ether, and recovering the same from this solution after washing it free from HCl.

In the case of *Cucurbita Maxima* seed fat, the mixed fatty acids of the oil were separated into different fractions of mean unsaturation by low-temperature crystallization from acetone and ether at —55°C. and —20°C., respectively (see crystallization chart). Three fractions, designated A, B, C in the increasing order of iodine value, were obtained. Their proportions are given in Table II. These fractions and the mixed fatty acids separated from the seed fat were analyzed spectrophotometrically (Table III).

TABLE I

Characteristics of Oils and Seeds

Seeds	(a) *C.* *Maxima*	(b) *L.* *Vulgaris*	(c) *C.* *Vulgaris*	(d) *B.* *Cerifera*
% Shell	32	60.1	54.1	52.3
% Kernel	68	39.9	45.9	47.7
% Oil (on kernel)	48	45.1	69.4	48.3
Oils				
Saponification Equivalent	300.7	301.6	298.2	301.3
Iodine Value (Wij's 30 min.)	98.3	126.5	116.2	126.8
Unsaponified and unsaponifiable matter, %	0.9	0.7	0.8	0.8
Free Fatty Acid (as Oleic) %	0.8	0.5	0.9	0.3
Refractive Index (at 40°C.)	1.4683	1.4711	1.4689	1.4715

MIXED FATTY ACIDS FROM *CUCURBITA MAXIMA* SEED FAT.
LOW-TEMPERATURE CRYSTALLIZATION CHART

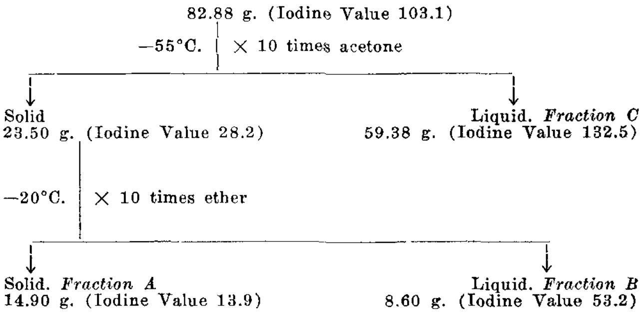

TABLE II

Fractions Obtained by Low-Temperature Crystallization of *C. Maxima* Mixed Fatty Acids (Iodine Value 103.1)

	Weight (g.)	% of Total	Iodine Value	Fraction
Soluble in acetone at —55°C.	59.38	71.6	132.5	C
Soluble in ether at —20°C.	8.60	10.4	53.2	B
Insoluble in ether at —20°C.	14.90	18.0	13.9	A
Total	82.88	100.0		

TABLE III

Spectrophotometric Data

	Iodine Value	$E^{1\%}_{1\,cm.}$ 268 mμ[b] 170°/15 min.	$E^{1\%}_{1\,cm.}$ 234 mμ 180°/60 min.
C. Maxima			
Mixed fatty acid[a]	103.1	3	396
Fraction A	13.9	nil	32
Fraction B	53.2	nil	146
Fraction C	132.5	4	532
L. Vulgaris			
Mixed fatty acid[a]	132.5	5	580
C. Vulgaris			
Mixed fatty acid[a]	123.0	5	531
B. Cerifera			
Mixed fatty acid[a]	132.6	5	564

[a] Excluding non-saponifiable matters.
[b] E values at 268 mμ being lower than 10 were neglected [see Hilditch and Shrivastava (4)].

Specific extinction coefficient values taken for calculation are according to Hilditch *et al.* (2):

Linoleic – $E^{1\%}_{1\,\mathrm{cm.}}$ at 234 mμ – 906
180°/60 min.

Linolenic – $E^{1\%}_{1\,\mathrm{cm.}}$ at 268 mμ – 555
170°/15 min.

Linolenic – $E^{1\%}_{1\,\mathrm{cm.}}$ at 234 mμ – 575
180°/60 min.

Oleic acid content (iodine value 90.1) was calculated by allowing for the contribution in the iodine value of the mixed fatty acids by linoleic acid. It was assumed that no palmitoleic acid was present in the various mixtures and all monounsaturated acids and diunsaturated acids were assumed to be oleic and linoleic acids, respectively.

Fraction A of *Cucurbita Maxima* seed fat, containing the most saturated acids, was esterified with methyl alcohol, and the methyl esters were fractionated in an E. H. P. column (*cf.* Longenecker). The saponification equivalents and iodine values of each of the fractions were determined (Table IV). The fatty acid composition of each fraction as computed from distillation and spectrophotometric data is given in Table V.

TABLE IV

Methyl Ester Fractionation Data for Fraction A of *C. Maxima* Seed Fat Acids

Fraction No.	Weight in g.	Saponification Equivalent	Iodine Value
A_1	2.82	275.6	6.4
A_2	4.84	284.6	10.1
A_3 (Residue)	1.30	331.0[a]	40.1

[a] Saponification Equivalent of the Fraction A_3, excluding non-saponifiables (detd.) 303.0; iodine value 37.3.

TABLE V

Component Fractions A, B, and C of *C. Maxima* Fatty Acids as Calculated from Spectrophotometric Data for Ester Fractionation Analyses (excluding non-saponifiables)

	A (18.0%)	B (10.4%)[a]	C (71.6%)[a]	Total
Palmitic	8.7	3.3	4.9	16.9
Stearic	7.3	2.7	4.0	14.0
Oleic	1.3	2.7	20.7	24.7
Linoleic	0.7	1.7	42.0	44.4

[a] Saturated acids in Fractions B and C were distributed between palmitic and stearic, assuming them to be in same proportion as in A [*cf.* Hilditch (13)].

For *Lagenaria Vulgaris, Citrullus Vulgaris,* and *Benincasa Cerifera* compositions of the seed fats were computed from spectrophotometric analyses only (Table VI).

TABLE VI

Component Fatty Acids of *L. Vulgaris, C. Vulgaris,* and *B. Cerifera* Seed Fats

Component Fatty Acids % by Wt. (excluding non-saponifiables)	(b) *L. Vulgaris*	(c) *C. Vulgaris*	(d) *B. Cerifera*
Saturated	17.8	22.7	15.8
Oleic	18.2	18.7	21.9
Linoleic	64.0	58.6	62.3

Data obtained in the present investigation on chemical composition of seed fats of *Cucurbita Maxima, Lagenaria Vulgaris,* and *Citrullus Vulgaris* have been compared with those previously reported in the literature (Table VII).

TABLE VII

Comparison of Previous Results with the Present Findings

	Saturated		Unsaturated	
	Palmitic %	Stearic %	Oleic %	Linoleic %
C. Maxima				
Jamieson and Baughmann (5) (Sub-tropic)	12.9	6.3	36.7	44.1
	19.2			
Present investigation (India)	16.9	14.0	24.7	44.4
	30.9			
L. Vulgaris				
Aggarwal and Dutt (6) (India)	ca. 50.0		ca. 50.0	
Present investigation (India)	17.8		18.2	64.0
C. Vulgaris				
Pieraerts (7) (Tropics)	31.0		43.0	26.0
Rankov and Popov (8) (Russia)	21.4		13.4	65.2
Rankov and Popov (9) (Russia)	21.6		14.7	63.7
Dhingra and Biswas (10) (India)	16.0		35.3	48.7
Nolte and Loesecke (11) (Florida)	15.7		13.5	70.8
Present investigation (India)	22.7		18.7	58.6
B. Cerifera				
Present investigation (India)	15.8		21.9	62.3
Phaduis and co-workers (12)	8.53	3.99	19.16	68.32
	12.52			

Discussion

Examination of the seed fats of *Cucurbita Maxima* has been reported earlier by Jamieson and Baughmann (5), and of *Lagenaria Vulgaris* by Aggarwal and Dutt (11). Both these investigations are dated prior to the introduction of the perfected technique of studying seed fat composition by spectrophotometric analysis and low-temperature fractionation. Naturally wide divergence has been noted between the reported data and those obtained in the present investigation.

In *Cucurbita Maxima* seed fat, deviation has been observed only in the amounts of saturated and oleic acids; the proportion of linoleic remained constant. The high percentage of saturated acids found in the present sample can perhaps be explained as being partly due to environmental factors for tropical conditions are considered likely to induce the formation of more saturated acids, and partly due to greater accuracy assured by the improved technique adopted.

In *Lagenaria Vulgaris* fat the quantity of saturated acids found in the present investigation is lower by 33% than that previously reported (6). At the same time the amount of unsaturated acids is correspondingly higher, the linoleic acid content is quite high (ca. 64%). The present results are however deemed to be more reliable.

The seed fat of *Citrullus Vulgaris* has been examined by several workers in different countries. The present results differ from all of these to a greater or less extent; the difference is particularly marked in the case of tropical samples reported by Pieraerts (7) and by Dhingra and Biswas (10). Among the tropical samples the percentage of linoleic acid is found in the present work to be highest, being about 30% higher than that reported by Pieraerts and about 10% higher than in the sample investigated by Dhingra *et al.* As a result the proportions of oleic acids are 24% and 16% higher, respectively, than those reported in the present investigation. The content of saturated acids shows a lack of uniformity. But again, it is obvious that the present results are likely to be more reliable than earlier ones owing to the limitations of the earlier analytical methods. The divergence from the Russian samples (8, 9) however is not wide. Here the linoleic acid content is higher than the present report by about 5 to 6%. This is compensated for by an equivalent lowering in oleic acid content. The proportion of saturated acids remains practically the same. The American sample (11) contains the maximum amount of linoleic acid, which is 10% higher than the present sample. The contents of oleic and saturated acids are lower in proportion. Besides the differences expected because of the methods of investigation followed, these results suggest the tendency of the composition of *Citrullus Vulgaris* seed fat to vary with environmental conditions.

The seed fat composition of *Benincasa Cerifera* has been reported from India by Phaduis *et al.* (12), who did not use spectrophotometric technique. The difference between the two results is not wide. The variation is confined to slightly higher content of linoleic acid (ca. 6%) and lower contents of saturated acid (ca. 3%) and oleic acid (ca. 2%), which in this case was probably due to adoption of older techniques.

It is evident from the results obtained that the component acids of these four allied members of the *Cucurbitaceae* family are of a more or less simple nature and do not contain triene acids like trichosanic or elaeostearic acid, which are found in some other members of this family.

The tendency toward variation in composition according to environmental conditions is also apparent. It should be found possible therefore to obtain linoleic-rich oils from the members, *Benincasa Cerifera, Lagenaria Vulgaris,* and *Citrullus Vulgaris,* by providing proper environments. All the seeds are rich in fat content, and, if available in commercial quantities, the oils would be quite suitable for edible and soap-making purposes. The composition of the seed fat of *Cucurbita Maxima,* as reported here, approaches that of Indian sesame oil and so this can be utilized as a substitute to fulfill certain purposes.

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A Comparison of Commercially Used Phenolic Antioxidants in Edible Animal Fats

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BUTYLATED hydroxyanisole (BHA) and propyl gallate, alone and in various combinations, are the most widely used food antioxidants today. Another phenolic antioxidant, butylated hydroxytoluene (BHT), was recently approved for food use by the Food and Drug Administration. BHT was first patented by Stevens and Gruse (6) in 1940 as a petroleum oil antioxidant and has since been used widely in the gasoline field. Seubert and Andrews (5) have shown that under accelerated conditions BHT at .01% concentration increased the stability of paraffin wax and polyethylene-paraffin wax mixtures tenfold.

Polister and Mead (4) report that when linoleic acid is exposed to ionizing radiation, a chain reaction similar to oxidation is introduced. They found however that when BHT was added in concentrations of .0003% and higher, oxidation was reduced to practically zero. Kraybill and Dugan (3) in reporting on new antioxidants stated that BHT was effective as an antioxidant for animal fats. They stated that, when used in lard, BHT was slightly more effective than BHA in increasing AOM stability but not as effective as BHA in carry-through stability as judged by the Schaal Oven Method. They also reported marked synergism between BHT and BHA in lard.

A purified form of BHT has recently been approved for food use by the Food and Drug Administration. Tappel (7) gives a very good comparison of the properties and general characteristics of BHA, propyl gallate, and nordihydroguaiaretic acid. Moore and Bickford (1) give an excellent study on the evaluation of 13 different antioxidants on the AOM stability of lard, cottonseed oil, and cottonseed shortening. They did not however include the carry-through stability effect of the different antioxidants or the effect of various combinations of these antioxidants. It was felt therefore that a basic study of BHA, BHT, and propyl gallate was needed so that a more intelligent approach could be made in utilizing the best characteristics of each of these three food antioxidants in preparing commercial formulations. This study should include a comparison of their physical characteristics, their effect on AOM stability in lard, and the effect of their carry-through stability in lard, as judged by the Schaal Oven Method. It should include the basic antioxidants as well as synergistic mixtures of the basic antioxidants with each other and with citric acid.

Physical Properties

Figure 1 shows a comparison of the structure and the physical properties of BHA, BHT, and propyl gallate. We have shown the structural formula for only the three isomer of BHA. All the tests reported herewith for BHA were made, using BHA which contained 95% or more of the three-isomeric form. Several investigators have reported that the three isomer