

THE HIGHER FATTY ACIDS OF POLLEN OF ZEA MAYS

Thesis for the Degree of M. S. MICHIGAN STATE UNIVERSITY Charles Richard Barr 1957 THESIS

C. Z.



Ì

MICHIGAN STATE UNIVERSITY

EAST LANSING, MICHIGAN

.

THE HIGHER FATTY ACIDS OF POLLEN OF ZEA MAYS

By

Charles Richard Barr

AN ABSTRACT

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

1957

CALBUC. Approved

Many articles have been published on the chemical composition of pollens. Analyses of corn pollen for minerals, amino acids, carbohydrates, and vitamins have been reported in the literature, but there has been no systematic study of the fatty acids.

An investigation was made of the higher fatty acids in the pollen of <u>Zea mays</u>. The lipids were separated from corn pollen by extraction with ethyl ether. Saponification, dilution with water, and extraction with Skellysolve "B" followed by acidification of the aqueous solution yielded the free fatty acids. The fatty acids were esterified, and the methyl esters distilled under reduced pressure using a Stedman column. The methyl esters of the fatty acids were collected in five fractions, and several physical constants were determined for each fraction.

The fatty acids in each fraction were identified by making the appropriate derivatives. The saturated fatty acids were identified by the melting points of the p-bromophenacyl esters, the mono-unsaturated fatty acids by the melting points of the dihydroxy acid, and the more unsaturated fatty acids by the melting points of the bromine addition products. A quantitative estimation of each fatty acid was made by substituting the determined iodine numbers, thiocyanogen numbers, and percentage of saturated fatty acids into empirical equations.

The following higher fatty acids were identified in corn pollen: palmitic, 21.6%; stearic, 1.1%; oleic, 7.5%; linoleic, 22.9%; and linolenic, 23.5%. The percentages listed indicate the relative abundance of each fatty acid of the total fatty acids obtained.

Palmitic and linolenic acids were found to be present in larger quantities in corn pollen than reported for other corn products; to the contrary, stearic, oleic, and linoleic acids were found in smaller quantities in corn pollen.

THE HIGHER FATTY ACIDS OF POLLEN OF ZEA MAYS

By

Charles Richard Barr

A THESIS

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

.

1957

7-14-57 22112

TABLE OF CONTENTS

INTR(ODU	CTI	ON	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	٠	٠	1
HIST	URI (CAL	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	3
EXPE	RIM	ENT.	AL	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	10
	Pro	epa	ra	tio	on	of	F	at	ty	A	ci	١d٤	3.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	11
	Pr	epa	ra	tio	on	ar	ıd	Fr	ac	ti	lor	at	tic	on	of	6 1	íe t	thy	1	Es	ste	rs	3.	•	•	•	•	•	•	•	•	1 1
	An	aly	ti	ca]	L	le t	hc	bds	ö.	•	•	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12
	Pr	epa	ra	ti	on	o	5 I)er	·iv	at	iv	es	5.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	ᅶ
RESU	LTS	AN	D	DIS	SCL	JSE	SIC)N	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	18
SUMM	ARY	, .	•		•	•	•	•	•	•	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	2 3
BIBL	IOGI	RAP	ΗY	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	25

LIST OF TABLES

TAELE		P.	AGE
I.	Mineral Content of Corn Pollen	•	4
II.	Amino Acid Content of Corn Pollen	•	6
III.	Carbohydrate Content of Corn Pollen	•	7
IV.	Vitamin Content of Corn Pollen	•	7
v.	Fatty Acid Content of Some Corn Products	•	9
VI.	Fractionation of Methyl Esters of Fatty Acids In Corn Pollen	•	20
VII.	Quantitative Distribution of Higher Fatty Acids		
	In Corn Pollen	•	21

AC KNOWLEDGMENTS

The author wishes to express his sincere thanks to Professors H. M. Sell and C. D. Ball for their interest and guidance throughout the course of this work. The writer deeply appreciates the financial support from the National Science Foundation. Grateful acknowledgment is also due Mrs. Jean Lagowski, Mrs. Audrey Anderson, and Mrs. Jean Brehmer for their assistance in the preparation of the manuscript. INTRODUCTION

-

ħ

INTRODUCTION

The important role played by pollen in the life cycle of plants has attracted the attention of scientists for many years in an attempt to learn the chemistry through which pollen manifests its physiological effects, culminating in fertilization. Due to its importance in the production of foodstuffs, for consumption by man, pollen is among the first of the natural products studied by modern chemists. Pollen research in recent years has been influenced largely by the fact that pollen may be responsible for certain allergies. Much of the recent literature in this field is devoted to studies of the causes of these allergies, particularly the component responsible for that allergy assumed to be produced by the pollen of ragweed.

The work done on pollen chemistry has been somewhat limited because of the difficulties experienced in collecting sufficient pollen samples for chemical analyses. Analyses of a large number of different pollens for specific materials have been reported in the literature, but only thirteen pollens have had relatively complete analysis (1).

The present study was made on the pollen of Zea mays, a highly important agricultural crop in this country. The pollen of this plant also lends itself more readily to hand collection than that from most other species of plants. The flower of Zea mays is of the incomplete type with a single, easily accessible stamen located at the top of the plant. The plant depends upon the wind to shake the pollen onto the stigma, thus the pollen must be produced in large quantities and easily shaken from the pollen sacs. HISTORICAL

.

HISTORICAL

A review of the literature suggests that a great deal of chemical analysis and research remains to be done on corn pollen. Lundén (2) has reviewed the recent literature on pollen chemistry and summarizes his major findings regarding the pollen chemistry of many plant species.

The ash content of corn pollen varies from 2.55 to 3.83%. The mineral composition of the pollen ash has been determined quantitatively (1, 3). The results of these analyses are shown in Table I.

TABLE I

MINERAL CONTENT OF CORN POLLEN

	Refer	ences
Mineral	(1)	(3)
K	26.31	35.58
Na		0.69
P	10.19	18.20
Ca	3.92	1.02
Mg	8.23	4.60
A l		0,22
Fe	0,05	0.25
S		0.59
Cl		0,80

(Percentage of ash)

The amino acids in Zea mays have been identified by chromatographic techniques (4), and several have been estimated quantitatively (4, 5). These results are tabulated in Table II. In the table, column 2 indicates the presence (+) or absence (-) of amino acids as determined by chromatographic techniques; columns 3 and 4 show the percentage of each amino acid of the crude protein in freshly collected pollen and. pollen that had been stored for one year, respectively. Column 5 shows the percentage of amino acid of the crude protein in sweet corn pollen, and column 6 gives the average amino acid content of crude protein of pollen for a number of plant species. Vinson (6) isolated from airdried pollen a protein behaving like a glutelin which contained 24.98% of the total nitrogen in the pollen. Also present were adenine, choline, and the free amino acids arginine, lysine, tyrosine, and β hydroxyglutamic acid. Miyake (7) has reported the presence of adenine and choline in corn pollen.

The carbohydrates of corn pollen have been reported to consist of starch, dextrin, sucrose, glucose, fructose, and hemicellulose, the latter hydrolyzing into glucose and xylose; free pentoses are probably absent (7). Some quantitative data on carbohydrates in corn pollen are given in Table III.

Redemann <u>et al</u>. (10) have identified quercetin as the major yellow pigment of the pollen of Zea mays.

Sagromsky (11) determined the thiamine content and Nielsen <u>et al</u>. (4) have determined a number of other B-vitamins present in <u>Zea</u> <u>mays</u>. Table IV summarizes the findings of these authors.

5

THE HIGHER FATTY ACIDS OF POLLEN OF ZEA MAYS

By

Charles Richard Barr

A THESIS

Submitted to the College of Science and Arts Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

•

1957

7-14-57 42112

TABLE OF CONTENTS

INTRO	ODUCT	ION	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	٠	٠	1
HIST	URICAI		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	3
EXPE	RIMENT	TAL	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	10
	Prepa	ara	tio	n	of	F	at	ty	A	lci	١d٤	3.	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	11
	Prepa	ira	tio	n	an	d	Fr	ac	ti	lor	at	ic	on	of	1	/iet	thy	' 1	E٤	ste	r	5.	•	•	•	•	•	•	•	•	11
	Analy	7 ti	cal	M	let	ho	ds	· •	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	12
	Prepa	ira	tio	n	of	D	er	iv	at	tiv	es	5.	•	•	•	•	•	•	•	•	•	•	•	•	•	٠	•	•	•	•	ᅶ
RESU	LTS AI	ND I	JIS	CU	SS	10	N	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	18
SUMM	ARY,	• •	•	•	•	•	•	•	•	•	•	٠	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	2 3
BIBL	IOGRAI	PHY	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	25

LIST OF TABLES

TAELE		PAC	ΞE
I.	Mineral Content of Corn Pollen	• 1	ŧ
II.	Amino Acid Content of Corn Pollen	. 6	Ś
III.	Carbohydrate Content of Corn Pollen	• 1	7
IV.	Vitamin Content of Corn Pollen	•	7
v.	Fatty Acid Content of Some Corn Products	• .	9
VI.	Fractionation of Methyl Esters of Fatty Acids	. 20	C
VII.	Quantitative Distribution of Higher Fatty Acids		
	In Corn Pollen	• 2	1

AC KNOWLEDGMENTS

The author wishes to express his sincere thanks to Professors H. M. Sell and C. D. Ball for their interest and guidance throughout the course of this work. The writer deeply appreciates the financial support from the National Science Foundation. Grateful acknowledgment is also due Mrs. Jean Lagowski, Mrs. Audrey Anderson, and Mrs. Jean Brehmer for their assistance in the preparation of the manuscript. INTRODUCTION

-

INTRODUCTION

The important role played by pollen in the life cycle of plants has attracted the attention of scientists for many years in an attempt to learn the chemistry through which pollen manifests its physiological effects, culminating in fertilization. Due to its importance in the production of foodstuffs, for consumption by man, pollen is among the first of the natural products studied by modern chemists. Pollen research in recent years has been influenced largely by the fact that pollen may be responsible for certain allergies. Much of the recent literature in this field is devoted to studies of the causes of these allergies, particularly the component responsible for that allergy assumed to be produced by the pollen of ragweed.

The work done on pollen chemistry has been somewhat limited because of the difficulties experienced in collecting sufficient pollen samples for chemical analyses. Analyses of a large number of different pollens for specific materials have been reported in the literature, but only thirteen pollens have had relatively complete analysis (1).

The present study was made on the pollen of Zea mays, a highly important agricultural crop in this country. The pollen of this plant also lends itself more readily to hand collection than that from most other species of plants. The flower of Zea mays is of the incomplete type with a single, easily accessible stamen located at the top of the plant. The plant depends upon the wind to shake the pollen onto the stigma, thus the pollen must be produced in large quantities and easily shaken from the pollen sacs. HISTORICAL

·

HISTORICAL

A review of the literature suggests that a great deal of chemical analysis and research remains to be done on corn pollen. Lundén (2) has reviewed the recent literature on pollen chemistry and summarizes his major findings regarding the pollen chemistry of many plant species.

The ash content of corn pollen varies from 2.55 to 3.83%. The mineral composition of the pollen ash has been determined quantitatively (1, 3). The results of these analyses are shown in Table I.

TABLE I

MINERAL CONTENT OF CORN POLLEN

	Refer	rences
ineral	(1)	(3)
K	26.31	35.58
Na		0.69
P	10.19	18,20
Ca	3.92	1.02
Mg	8.23	4.60
Al	**	0,22
Fe	0 .05	0.25
S		0.59
Cl		0,80

(Percentage of ash)

The amino acids in Zea mays have been identified by chromatographic techniques (4), and several have been estimated quantitatively (4, 5). These results are tabulated in Table II. In the table, column 2 indicates the presence (+) or absence (-) of amino acids as determined by chromatographic techniques; columns 3 and 4 show the percentage of each amino acid of the crude protein in freshly collected pollen and pollen that had been stored for one year, respectively. Column 5 shows the percentage of amino acid of the crude protein in sweet corn pollen, and column 6 gives the average amino acid content of crude protein of pollen for a number of plant species. Vinson (6) isolated from airdried pollen a protein behaving like a glutelin which contained 24.98% of the total nitrogen in the pollen. Also present were adenine, choline, and the free amino acids arginine, lysine, tyrosine, and β hydraxyglutamic acid. Miyake (7) has reported the presence of adenine and choline in corn pollen.

The carbohydrates of corn pollen have been reported to consist of starch, dextrin, sucrose, glucose, fructose, and hemicellulose, the latter hydrolyzing into glucose and xylose; free pentoses are probably absent (7). Some quantitative data on carbohydrates in corn pollen are given in Table III.

Redemann <u>et al</u>. (10) have identified quercetin as the major yellow pigment of the pollen of Zea mays.

Sagromsky (11) determined the thiamine content and Nielsen <u>et al</u>. (4) have determined a number of other B-vitamins present in Zea mays. Table IV summarizes the findings of these authors.

5

TABLE II

AMINO ACID CONTENT OF CORN POLLEN

(Percentage of crude protein)

Amino Acid	Zea	mays (5)	Sweet Corn	Average Plant
	Present	Fresh	Aged	Pollen (4)	Pollen (8)
Crude Protein (N.6.25)		25.63	26 .25	26.98	26 .34
Arginine	+	6.3	5.7	4.7	5.3
Histidine	+			1.5	2.5
Lysine	+	5.9	5.0	5.7	6.1
T ry ptoph an	+	0.5	0.6	1,6	1.4
Phenylalanine	+	2.9	2.3	3.5	4.1
Cystine	+			0.6	-
Methionine	+	1.6	1.5	1.7	1.9
Threonine	+			4.6	4.1
Leucine	+	7.5	5.6	5.6	7.1
Isoleucine	+			4.7	5.1
Valine	+		-	6.0	5.8
Glutamic Acid	+			9.1	
Olycine	+				
Alanine	+				
Serine	+				
Aspartic Acid	+				
Proline	+				
Hydrexypreline	+				
Tyresine	+	1.9	1.9		
a-Amine Butyric Acid	+				

TABLE III

CARBOHYDRATE CONTENT OF CORN POLLEN

(Percentage of pollen)

		References	3
Carbohydrate	(1)	(3) ¹	(9)
Starch	22.40	16.04	16.19
Dextrin			0,50
Reducing Sugar (as Glucose)	6.88	4.61	0,59
Non-reducing Sugar (as Sucrose)	7.31	8.74	7.80
Pentosans			5.73

¹Average of three varieties.

TABLE IV

VITAMIN CONTENT OF CORN POLLEN

 $(\mu g_{\bullet}/g_{\bullet} \text{ of pollen})$

The second se	Refe	rences
Vitamin	(4)	(11)
Thiamin		1.4-7.9
Riboflavin	6.2	
Nicotinic Acid	71.8	
Pyridoxine	12.7	
Pantothenic Acid	5.5	
Biotin	0.55	
Inositol (mg./g.)	30	

¹Variation for seven different pollens.

Paton (12) has determined the enzymes present in a number of plants and has reported the following to be present in corn pollen: amylase, invertase, catalase, reductase, pectinase, trypsin, and pepsin.

A phytosterol and an inositol were isolated from corn pollen by Miyake (9). Anderson (13) isolated a phytosterol palmitate, which upon hydrolysis gave two different phytosterol fractions, a saturated hydrocarbon (apparently <u>n</u>-nonacosane), a C₃₀-saturated alcohol, and a phosphatide.

The lipid fraction of pollens other than that of Zea mays has been studied; Heyl (14) found the following acids in ragweed pollen: formic, acetic, valeric, lauric, an unsaturated acid $C_{10}H_{10}O_2$, oleic, linoleic, palmitic, and myristic. Kiesel and Rubin (15) reported a high content of heptacosane in the crude lipid from sugar beet pollen. In hazel pollen, Sosa and Sosa-Bourdouil (16) noted the presence of palmitic acid, one C_{12} -acid, tricosane, hexadecanol, and two unsaturated sterols. Mariella <u>et al</u>. (17) reported analyses and approximate empirical formulae for seven compounds isolated from the nonsaponifiable fraction of ragweed pollen. From the pollen cement of <u>Lilium candidum</u>, Tappi and Monzami (18) have isolated heptacosane, phytofluene, and γ -sitosterol.

Redemann (19) has indicated the presence of a growth regulator in the ethyl ether soluble fraction of corn pollen. Even though the fatty acids of several corn products have been reported, as shown in Table V, those of corn pollen have received little attention.

The work described in this thesis consists of the analysis and identification of the higher fatty acids in the lipid fraction of corn Pollen.

8

TABLE V

FATTY ACID CONTENT OF SOME CORN PRODUCTS

			011		Corn	Corn	Corn
Fatty Acid			ences		erm Oil		Grain
	(20) ¹	(21) ¹	(22)1	(23) ²	(24)	(25)	(26)
Myristic	0 .] .			1.7	0.2		
Palmitic	8.1	7.8	7.8	11.0	13.0	21.2	8.0-8.7
Stearic	2.5	3.6	3.5	2.9	0.9	7.8	4.1-5.1
Arachidic	0.0	0 li	0-1:	-	1.5		
Behenic	3				0•2		
Eicosenoic		••			1.5		
Lignoceric	0.0	0.2	0.2	-			
Palmitoleic	1.2			1.6	0.2		
Oleic	30 .].	46.3	46.3	48.8	41.9	37•7	31.0-31.9
Linoleic	56.3	41.7	41.8	34.0	40.6	31.1	53.8-56.7
Linolenic		-				1.2	

(Percentage of product)

1 Expressed as weight percent.

a Expressed as mole percent.

³Acids above C₁₈--1.7 percent.

EXPERIMENTAL

..

EXPERIMENTAL

Preparation of Fatty Acids

The lipid fraction used in this study was extracted from corn pollen (variety <u>Golden Cross Bantam</u>) with ethyl ether in 1945 (19) and had been stored at temperatures below 0° C. The lipids (214.1 g.) were saponified with 31.6 g. of potassium hydroxide in 400 ml. of 95% ethanol by refluxing for 8 hours on a steam bath. After diluting the solution with 1600 ml. of distilled water, the unsaponifiable material was removed by extraction with Skellysolve "B". The Skellysolve "B" solution was in turn extracted several times with distilled water, and the water extracts combined with the ethanol-water phase. The ethanol-water solution, which contained the potassium salts of the fatty acids, was acidified with 40 ml. of concentrated hydrochloric acid, and the liberated fatty acids were extracted with ethyl ether. The ether was removed <u>in vacuo</u> (15 mm.) at 35° C. in an atmosphere of carbon dioxide. A residue of 112.4 g. of fatty acid remained in the flask. The Saponifiable fraction represented 49% of the original lipid material, while 51% consisted of unsaponifiable materials.

Preparation and Fractionation of Methyl Esters

Preparation of Methyl Esters of Fatty Acids. A solution of 112.4 g. of the fatty acids in 250 ml. of absolute methanol containing 5% sulfuric acid (by weight) was refluxed for 6 hours on a steam bath. The methanol was removed from the methylated fatty acids <u>in vacuo</u> (15 mm.) at 40° C. The residual oil was diluted with water and neutralized with a 10% sodium carbonate solution. The methyl esters were extracted with ethyl ether, and the ether solution was washed with water to remove traces of sodium carbonate. After removal of the ether <u>in vacuo</u> (15 mm.) at 35° C., 105 g. of crude methyl esters was recovered.

Fractionation of Methyl Esters of Fatty Acids. A Stedman packed column constructed of Pyrex glass which was 0.762 inch in diameter and 24 inches long, and contained Stedman No. 104 packing, was used in this study. The column was centered in a 42 mm. (i.d.) Pyrex tube by wrapping both ends of the column with asbestos tape. A nichrome wire helix was placed around the tube to permit the column to be heated electrically. The heating jacket was insulated, and protected by another Pyrex tube of 64 mm. (i.d.). The 250 ml. flask containing the methyl esters was heated by a Glas-Col heating mantle. The temperatures of the flask and column was regulated by means of A. C. variable transformers. A still head with an enclosed thermometer was used at the top of the column and the reflux ratio was regulated by a stopcock located below the condenser. The crude mixture of the methyl esters of the fatty acids was fractionated in the column previously described. Five fractions of the methyl esters were collected between 155-184^o C./2-3 mm. at a reflux ratio of 10:1.

Analytical Methods

Iodine Absorption Number (Wijs Method) (27). A sample of 0.2 g. of methyl ester or fatty acid was weighed and transferred to a 500 ml. glass-stoppered iodine flask containing 20 ml. of carbon tetrachloride (C.P.). Twenty-five milliliters of iodine-chlorine solution was added from a pipette and the reaction flask allowed to stand in the dark for 30 minutes. At the end of this period 20 ml. of 15% potassium iodide solution and 100 ml. of recently boiled and cooled water were added.

ţ

The liberated excess iodine was titrated with a 0.1 <u>N</u> sodium thiosulphate solution using starch indicator. Two blank determinations were made. <u>Preparation of Reagent</u>. Washed and dried chlorine gas was passed into a solution of 13 g. of iodine in 1 l. of glacial acetic acid (99.5%) until thiosulphate titration of the solution was approximately doubled.

Iodine No. =
$$[M1. of S_2O_3(blank)] - [M1. of S_2O_3(sample)](N)(126.91)(100)(G. of sample)(1000)$$

<u>Refractive Index</u> (27). The refractive indices were determined with an Abbe refractometer at 25° C. using the D line of sodium.

<u>Saponification Equivalent</u> (28). A mixture of 0.4 g. of the methyl ester and 15 ml. of 3 <u>N</u> alcoholic sodium hydroxide solution was refluxed in a 150 ml. Erlenmeyer flask for 1 1/2 hours. After cooling, the excess alkali was titrated with 0.1 <u>N</u> hydrochloric acid solution to a phenolphthalein end point.

Saponification Eq. =
$$(G. \text{ of sample })(1000)$$

(M1. of NaOH)(N)-(M1. of HC1)(N)

<u>Thiocyanogen Number</u> (27). A sample (0.1 to 0.5 g.) of fatty acid, obtained by the saponification of the methyl ester, was dissolved in 25 ml. of thiocyanogen solution, and placed in the dark for 24 hours. Ten milliliters of 20% potassium iodide was added; after diluting with 100 ml. of water, the excess iodine was titrated with a 0.1 <u>N</u> sodium thiosulfate solution, using starch indicator. Blank determinations were made.

<u>Preparation of Reagents</u>. Thiocyanogen solution (0.2 N) was prepared by dissolving 8.4 g. of dry bromine in 100 ml. of anhydrous carbon tetrachloride, and diluting to 250 ml. with glacial acetic acid. Glacial acetic acid (250 ml.) was added to 25 g. of anhydrous, reagent grade lead thiocyanate in a 1 l. glass stoppered bottle. The bromine solution was added in small quantities and shaken after each addition until the bromine was decolorized. When addition was complete, the suspension was allowed to settle, the solution was filtered into a dry, brown, glassstoppered bottle and stored in the refrigerator.

SCN No. =
$$[M1. of S_2O_3(blank)] - [M1. of S_2O_3(sample)] (N)(126.9)(100)(G. of sample)(1000)$$

Quantitative Determination of Fatty Acids (30). A quantitative estimation of unsaturated fatty acids was made by substituting the experimentally determined iodine number, thiocyanogen number, and percentage of saturated fatty acids in the following equations.

In the absence of linolenic acid: % Oleic acid = 2.421(T.V.)-1.293(I.V.) % Linoleic acid = 1.194(I.V.)-1.202(T.V.) In the presence of linolenic acid: % Oleic acid = 1.6146(T.V.)-1.2275(I.V.)-0.6617(S)+66.17 % Linoleic acid = 1.3565(I.V.)-3.2048(T.V.)-1.6423(S)+164.23 % Linolenic acid= 1.5502(T.V.)-0.1290(I.V.)+1.3040(S)-130.40 T.V. = Thiocyanogen number I.V. = Iodine number S = % Saturated fatty acid

Preparation of Derivatives

Lead Salt-Ether Method of Separating Saturated and Unsaturated Fatty Acids (27). To 5 g. of fatty acid in a 100 ml. Erlenmeyer flask was added 15 ml. of ethanol and sufficient 15% potassium hydroxide solution to produce a distinct pink color with phenolphthalein. The soap solution was added cautiously to 60 ml. of 10% lead acetate solution (hot), and the flask rinsed with small portions of ethanol and hot

water. The solution was boiled gently for 5 minutes, shaken, cooled, and the flask rotated to cause the precipitated lead salts to adhere to the walls. After cooling, the supernatant liquid was decanted and the precipitate was washed with cold water. The flask was drained for 10 minutes and moisture removed from the soaps with filter paper strips. Sixty milliliters of ethyl ether was added and the lead salts disintegrated or dissolved by gently refluxing the ethereal suspension. The walls of the flask were rinsed with sufficient ethyl ether to bring the final volume to 75 ml. After cooling the ethereal solution in a refrigcrator for 15 hours, the precipitate was collected quantitatively on a Buchner funnel: the precipitate was washed with ethyl ether and dried thorough-Ly by suction. The precipitate was transferred immediately to a 100 ml. separatory funnel containing 25 ml. of ethyl ether and the filter paper was placed in a small flask. The lumps of lead salt were dispersed by shaking and allowed to settle. Ten milliliters of hydrochloric acid (2:1) was added, and the solution shaken for 2 minutes to decompose the lead soaps. The precipitate adhering to the filter paper was decomposed by the addition of 3 ml. of hydrochloric acid (2:1) and washed into the Separatory funnel with ethyl ether and water. After shaking, the contents of the separatory funnel were allowed to stand for 10 minutes and the aqueous solution drained off. The ether layer was washed with 15 ml. Portions of water until free of hydrochloric acid. After drying with 1 g. of anhydrous sodium sulfate, the ethereal solution was filtered into a tared 100 ml. flask. The sodium sulfate was washed with several small portions of anhydrous ethyl ether and the ether washings combined with the ether extract. The ether was removed at 35° C./15 mm. and the acids dried to constant weight at 110° C.

The ether solution containing the soluble lead salts was transferred quantitatively from the filter flask to a 100 ml. separatory funnel. A solution of 15 ml. of hydrochloric acid in h0 ml. of water was added and the separatory funnel shaken for 2 minutes. After standing for 10 minutes, the aqueous suspension of lead chloride was drained into a beaker. The lead chloride was allowed to settle, the supernatant liquid decanted, and the lead chloride washed with a small portion of ethyl ether. The e ther washings were combined with the contents of the separatory funnel, rotated, and allowed to stand for 10 minutes. The aqueous solution was separated and the ether layer washed with 25 ml. portions of water until free of hydrochloric acid. The ether layer was transferred to a tared flask and the ether removed at 35° C./15 rm. The residue in the flask was dried at 110° C. in a carbon dioxide atmosphere for 1 hour and the weight of residue determined by difference.

Preparation of p-Bromophenacyl Esters of the Saturated Fatty Acids (28). A suspension of 1 g. of fatty acid in 5 ml. of water in a small flask was neutralized with a 10% sodium hydroxide solution to a phenolphthalein end point and then made slightly acid to litmus. One gram of p-bromophenacyl bromide in 10 ml. of ethanol was added and the mixture heated under reflux for 3 hours. If a solid separated during refluxing, sufficient ethanol was added to effect solution. After cooling, the Precipitated ester was recrystallized from ethanol until a constant melting point was attained.

Preparation of Dihydroxy Derivatives of Mono-unsaturated Acids (29). One gram of acid and 1 g. of sodium hydroxide were added to 100 ml. of Water and the mixture warmed on the water bath until solution was effected. The solution of the sodium salt of the acid was ccoled and diluted with 300 ml. of ice-cold water, and 80 ml. of a 1% potassium permanganate solution was added quickly with stirring. After 5 minutes, the liquid was decolorized with sulfur dioxide, and 30 ml. of concentrated hydrochloric acid was added. The white flocculent precipitate of crude solid dihydroxy saturated acid was collected on a Buchner funnel, washed with 10 ml. of ethyl acetate, and air dried. The filter cake was mixed with 30 ml. of warm ethyl acetate, cooled, and filtered; then the acid was washed with several small portions of cold ethyl acetate. The acid was recrystallized to a constant melting point.

Preparation of Bromo-derivatives of Unsaturated Fatty Acids (30). One gram of fatty acid was dissolved in 25 ml. of ethyl ether and the solution chilled to 0° C. in an ice bath. Bromine was added at such a rate that the temperature did not exceed 20° C. and until a deep red color persisted. The reaction mixture was placed in the refrigerator overnight, and the solid white precipitate was collected on a Buchner funnel.

The brominated acids were separated on the basis of their different Solubilities in organic solvents. The dibromo-acid is soluble in Petroleum ether, while the tetrabromo-acid, insoluble in petroleum ether, is soluble in ethyl ether. The hexabromo-acid, insoluble in petroleum ether and ethyl ether, is soluble in hot benzene. The melting points of the brominated acids were determined.

17

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

The saponifiable material in corn pollen represented 4% of the total ether extract, while the remaining 51% consisted of unsaponifiable material.

The results of fractionation of the methyl esters formed by esterification of the saponifiable material in corn pollen are summarized in Table VI. Some physical and chemical characteristics of the 5 fractions collected are given. The fatty acids identified and their derivatives in each fraction are listed. The saturated fatty acids identified were palmitic and stearic, while the unsaturated acids were oleic, linoleic, and linolenic.

The quantitative values of the fatty acids in corn pollen are shown in Table VII. Calculation of the percentage of each acid in the distillate gave the following results: palmitic, 21.6%; stearic, 1.1%; oleic, 7.5%; linoleic, 22.9%; and linolenic, 23.5%.

A comparison of these values with those tabulated for corn oil, corn germ oil, corn starch, and corn grain, in Table V, shows that stearic, oleic, and linoleic acids were present in smaller quantities in corn pollen, whereas palmitic and linolenic acids were present in larger quantities. Of these differences, that of linolenic acid was outstanding. This acid was approximately 20 times more abundant in corn pollen than in corn starch and the only corn product in which it has been re-Ported. The unsaturated fatty acids in corn pollen were found to represent 53.% of the fatty acids and were lower than those values found for the

ΙΛ	
TABLE	

FRACTIONATION OF THE METHYL ESTERS OF THE FATTY ACIDS IN CORN POLLEN

Fraction number	in degrees C. at 2-3 mm. pressure ^{1,2}	Weight of fraction in grams	Iodine number	Refractive ind g x at 25 C.	equiv meth Four	equivalents of methyl esters Found Calcd.	of den degn Ubservec	of derivative, degrees C. Observed ² Reported	Acid identified
Original methyl ester	ster	100.2	21,2	1 . 468	293	ł	ł	ł	8
ч	155-162 (155)	10.1	20	ינוווי.ר	262	270.5	86 ³	86	Palmitic
2	162-168 (165)	17.1	80	1.450	280	(270.5 (294.5	(36 ° 111	(86 (114.7-115.2	Palmitic 2 Linoleic
m	168-176 (174)	16.9	128	1,455	1 82	(270.5 (296.5 (294.5	(86 (132 (132 (132	(86 (132 (1.7-7.4LL)	Palmitic Oleic 2 Linoleic
7	176-180 (177)	23.5	712	1.468	1 52	(298.5 (296.5 (292.5	(90 ³ (132 4 (181 ⁶	(90 (132 (181.5-181.6	Stearic Oleic 6 Linolenic
м	(781) 781-081	L. ?	210	1.465	293	(298.5 (294.5 (292.5	(90 ³ (114 ⁶ (181 ⁶	(90 (114.7-115.2 (181.5-181.6	Stearic 2 Linoleic 5 Linolenic
Residue	!	23.6	ł	ł	ł	ł	ł	:	8

³<u>p</u>-Bromophenacyl ester (28).

⁴Hydroxy acid (33).

⁵Tetrabromo addition compound (31).

TABLE VII

QUANTITATIVE DISTRIBUTION OF HIGHER FATTY ACIDS IN CORN POLLEN

			Fraction	<u></u>		
	1	2	3	4	5	Total
Weight in Grams	9 .5	16.3	15.8	22.0	8.7	72.3
Iodine Number	21.1	84.6	134.4	227.9	220.5	
Thiocyanogen Number	15.4	<u>ц</u> ,2	7 9 . 5	141.6	132.0	
Percent Saturated Fatty Acid	89 .3	51.7	21.6	2.6	5.0	
Palmitic Acid						
Percent	89 .3	51.7	21.6			
Grams	8.51	8.43	3.41			20.35
Stearic Acid						
Percent				2.6	5.0	
Grams		~ •		0.57	0.44	1.01
Oleic Acid						
Percent	3.1	0.4	ът•8	16.9	7.9	
Grams	0.30	0.07	2.34	3.72	0.69	7.12
Linoleic Acid						
Percent	7.6	47.9	56.6	8.2	27.0	
Grams	0.72	7.80	8.95	1.81	2.34	21.62
Linolenic Acid						
Percent			7.0	72.3	60.1	
Grams			1.10	15.90	5.23	22.23

other corn products previously mentioned. The high percentage of linolenic acid may be of considerable significance, since the unsaturated fatty acids may be more easily oxidized and metabolized by pollen during growth of the pollen tube than are the other fatty acids. If these acids are metabolized, the unsaturated fatty acids could serve as a better source of nutrients for the growing part of the pollen tube. SUMMARY

SUMMARY

A study was made of the saponifiable fatty acid fraction of pollen of Zea mays.

The fatty acids extracted from corn pollen were methylated and fractionated in a Stedman column. Some chemical and physical properties were determined.

Palmitic and stearic acids were identified as the <u>p</u>-bromophenacyl derivatives, and oleic, linoleic, and linolenic acids as the hydroxy and bromine addition compounds.

The percentage of each acid in the saponifiable fraction was: palmitic, 21.6%; stearic, 1.1%; oleic, 7.5%; linoleic, 22.9%; and linolenic, 23.5%.

A higher percentage of linolenic acid was found in corn pollen than was reported for corn oil, corn germ oil, corn starch, and corn grain. BIBLIOGRAPHY

BIBLIOGRAPHY

- 1. F. E. Todd and O. Bretherick, The Composition of Pollens, J. Econ. Entomol. <u>35</u>, 312 (1942).
- 2. R. Lunden, A Short Introduction to the Literature on Pollen Chemistry, Svensk Kem. Tidskr, 60, 201 (1954).
- 3. R. J. Anderson and W. L. Kulp, Analysis and Composition of Corn Pollen. Preliminary Report, J. Biol. Chem., <u>50</u>, 433 (1922).
- 4. N. Nielsen, J. Grommer, and R. Lunden, Investigations on the Chemical Composition of Pollen from Some Plants, Acta Chem. Scand., <u>9</u>, 1100 (1955).
- 5. B. C. Ray Sarkar, S. H. Wittwer, R. W. Luecke, and H. M. Sell, Quantitative Estimation of Some Amino Acids in Sweet Corn Pollen, Arch. Biochem., 22, 353 (1949).
- C. G. Vinson, Some Nitrogenous Constituents of Corn Pollen, J. Agr. Research, <u>35</u>, 261 (1927).
- 7. S. Miyake, Chemical Studies of Corn Pollen. II. Carbohydrates and Organic Bases, J. Biochem. (Japan), 3, 169 (1924).
- 8. N. Weaver and K. A. Kuiken, Quantitative Analysis of the Essential Amino Acids of Royal Jelly and Some Pollens, J. Econ. Entomol., <u>14</u>, 635 (1951).
- 9. S. Miyake, Chemical Studies of Corn Pollen. I. Isolation of Phytosterol and Inositol, J. Biochem. (Japan), 2, 27 (1922).
- 10. C. T. Redemann, S. H. Wittwer, C. D. Ball, and H. M. Sell, The Occurrence of Quercetin in the Pollen of Zea mays, Arch. Biochem., 25, 277 (1950).
- H. Sagromsky, Determination of the Vitamin B₁ Content of Pollen by the Phycomyces Test, Biol. Zentr., <u>66</u>, 140 (1947; C.A., <u>42</u>, 3030 (1948).
- 12. J. B. Paton, Enzymes of Pollen, Proc. Soc. Exptl. Biol. Med., <u>17</u>, 60 (1919).
- R. J. Anderson, Composition of Corn Pollen. II. Concerning Certain Lipoids, a Hydrocarbon, and Phytosterol Occurring in the Pollen of White Flint Corn, J. Biol. Chem., <u>55</u>, 611 (1923).

- 14. F. W. Heyl, Some Constituents of Ragweed Pollen, J. Am. Pharm. Assoc., 12, 669 (1923); C.A., 18, 1007 (1924).
- 15. A. Kiesel and B. Rubin, Untersuchungen uber pflanzliche Fortpflanzungszellen, Z. physiol. Chem., 182, 241 (1929).
- 16. A. Sosa and C. Sosa-Bourdouil, Études de quelques constituents chemiques du pollen de Corylus avellana L., Compt. rend., 235, 971 (1952).
- R. P. Mariella, T. B. Bernstein, and A. L. Mosher, Some Purification Studies on Ragweed Pollen, Bol col. quim. Puerto Rico, <u>9</u>, 15 (1952); C.A., <u>47</u>, 1331 (1953).
- 18. G. Tappi and A. Monzani, Constituents of the Pollen Cement of <u>Lilium Candidum</u>, Gazz. chim. ital., <u>85</u>, 725 (1955); C.A., <u>49</u>, 14922 (1955).
- 19. C. T. Redemann, Biochemical Studies of Pollen from <u>Zea mays</u>, Ph.D. Thesis, Michigan State College (1949).
- 20. F. J. Baur, Jr. and J. B. Brown, The Fatty Acids of Corn Oil, J. Am. Chem. Soc., <u>67</u>, 1899 (1945).
- W. F. Baughman and G. S. Jamieson, The Chemical Composition of Corn Uil, J. Am. Chem. Soc., <u>43</u>, 2696 (1921).
- 22. K. H. Kahler, The Preliminary Processing of Corn with Special Consideration of Biochemical Factors, Getreide, Mehl u. Brot, <u>1</u>, 24 (1947); Chem. Zentr., <u>1947</u> <u>II</u>, 1187; C.A., <u>43</u>, 5875 (1949).
- 23. H. E. Longenecker, Deposition and Utilization of Fatty Acids. II. The Non-preferential Utilization and Slow Replacement of Depot Fat Consisting Mainly of Oleic, and Linoleic Acids; and a Fatty Acid Analysis of Corn Oil, J. Biol. Chem., <u>129</u>, 13 (1939).
- 24. A. D. Fortunato, Chemical Composition of an Argentine Corn-Germ Oil, Industria y quimica, <u>11</u>, 132 (1949; C.A., <u>45</u>, 370.
- 25. J. W. Evans and D. R. Briggs, The Lipids of Corn Starch, Cereal Chem., <u>18</u>, 443 (1941).
- 26. F. A. Kummerow, The Composition of the Oil Extracted from 14 Different Varieties of Andropagon Sorghum var. vulgaris, Oil and Soap, 23, 273 (1946); C.A., 43, 2450 (1949).
- W. Horwitz, Editor, Official Methods of Analysis of the Association of Official Agricultural Chemists, eighth edition, Washington, D.C., A. O. A. C. (1955), pp. 465, 460, 469.

- 28. R. L. Shriner and R. C. Fuson, The Systematic Identification of Organic Compounds, New York, John Wiley and Sons, Inc. (1948), pp. 134, 157, 223.
- 29. A. Lapworth and E. N. Mottram, Oxidation Products of Oleic Acid. Part I. Conversion of Oleic Acid into Dihydroxystearic Acid and the Determination of the Higher Saturated Acids in Mixed Acids from Natural Sources, J. Chem. Soc., <u>127</u>, 1628 (1925).
- 30. H. K. Dean, Utilization of Fats, New York, Chemical Publishing Co. of N. Y., Inc. (1938), p. 61.
- 31. J. W. McCutcheon, Linoleic Acid, Organic Syntheses, Coll. Vol. 3, New York, John Wiley and Sons, Inc. (1955), p. 527.
- 32. -----, Linolenic Acid, Organic Syntheses, Coll. Vol. 3, New York, John Wiley and Sons, Inc. (1955), p. 532.
- 33. G. S. Jamieson, Vegetable Fats and Oils, New York, Reinhold Publishing Corp. (1943), p. 345.

WATSTRY LIEBANC

l

•

•

Date Due					
·					
			1		
Demco-293		l			

MICHICAN STATE UNIVERSITY OF ACADEMIDIAL AND APPIND SOCIAGE DEFAILTMENT OF CMEMISTRY EAST LANSING, MICHIGAN

Thesis Charles M.S. Barr. Charles 1057 The higher fatty acids of c.2 Zea mays.

