

To the Graduate Council:

I am submitting herewith a thesis written by Tae Hyong Rhew entitled "Triglyceride Composition of Naked-Seeded Pumpkin Seed Oil." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology and Science.

Sharon L. Melton
Sharon L. Melton, Major Professor

We have read this thesis
and recommend its acceptance:

Lugh Daynes
J. L. Collins

Accepted for the Council:

L. Evans Beth
Vice Chancellor
Graduate Studies and Research

Ag-VetMed

Thesis

79

.R449

cop. 2

TRIGLYCERIDE COMPOSITION OF NAKED-SEEDED PUMPKIN SEED OIL

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Tae Hyong Rhew

December 1979

1405032



ACKNOWLEDGMENTS

The author expresses sincere appreciation to his major professor and chairman of his graduate committee, Dr. Sharon L. Melton, for her tremendous help, knowledgeable guidance and encouragement throughout every stage of the research program. Appreciation is also offered to Dr. H. O. Jaynes and Dr. J. L. Collins for their help in participating as committee members.

The author is grateful to Dr. Miles, Head of the Department of Food Technology and Science, and the faculty of the department of Food Technology and Science for the help throughout the author's graduate studies.

He wishes to thank all of the assistants who helped with the research for this thesis.

Finally, the author wishes to express appreciation to his wife for her patience, encouragement, understanding and help.

ABSTRACT

The triglyceride (TG) structure of naked-seeded pumpkin seed oil (Cucurbita pepo, var. Lady Godiva) was determined. Crude oil of naked-seeded pumpkin seed was extracted from dried, ground whole seeds, alkali refined, bleached and deodorized. The oil was separated into four fractions by low temperature crystallization at 10 degree intervals from -30 to 0°C. The refined oil and oil in each fraction were hydrolyzed by pancreatic lipase, and the 2-monoglyceride was isolated by thin layer chromatography. Fatty acid composition of the refined oil, the oil in each fraction and the respective 2-monoglyceride were determined by gas chromatography. The fatty acid composition in the refined oil was 14.96% palmitic, 4.78% stearic, 49.11% oleic, 30.87% linoleic and 0.28% linolenic. Percentages of alignment structure of the triglycerides molecules (ASTM) of the oil from the naked-seeded pumpkin seed were calculated to be: SSS = 0.28, SSU = 1.63, SUS = 6.42, USU = 2.32, SUU = 36.75 and UUU = 52.88; where S = saturated fatty acid and U = unsaturated fatty acid. The main glycerides of the oil where S = saturated, O = oleic and U = linoleic plus linolenic were found to be SOO = 13.82%, OOU = 13.32%, OOO = 12.38%, OUU = 10.59% and OUS = 10.99%. The oil on fractionation by low temperature crystallization yielded two major fractions, C fraction at -10°C representing 27.95% of total oil, and D fraction, liquid at 0°C representing 44.4% of the total oil. In ASTM, fraction C had larger amounts of UUU, USU and SSU, and fraction D had larger amounts of SUU, SUS and SSS. The triglyceride composition was confirmed by quantitative fractional crystallization of the refined oil and by triglyceride determination in each fraction and calculation

of the original triglycerides from the triglycerides of each fraction.



TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. LITERATURE REVIEW.	3
Brief History of TG Structure Determination	3
Methods Used to Determine Triglyceride Structure	4
TG Composition of Some Fats	9
Pumpkin Seed Oil Characteristics	10
III. MATERIAL AND METHODS	14
Source of Naked-Seeded Pumpkin Seeds	14
Treatment of Naked-Seeded Pumpkin Seeds after Storage	14
Quantitative Fractional Crystallization at Low	
Temperatures	17
Separation of TGs	18
Preparation of 2-Monoglycerides	19
Fatty Acid Determination of TGs and 2-Monoglycerides	20
Calculation of Iodine Value	21
Calculation of TG Structure	21
IV. RESULTS AND DISCUSSION	23
Extracted Oil Properties	23
Oil Fractionation	23
Fatty Acid Composition of TG and 2-Monoglyceride	26
V. SUMMARY.	32
LIST OF REFERENCES	33
APPENDIXES	40
APPENDIX 1. BLEACHING OF OIL	41
APPENDIX 2. REFINING OF OIL	43
APPENDIX 3. CALCULATION OF IODINE VALUES	45
APPENDIX 4. SAMPLE CALCULATION OF MOLE PERCENTAGE OF	
EACH FATTY ACID	48
APPENDIX 5. CALCULATIONS FOR COMPUTED FATTY ACID	
COMPOSITION OF REFINED OIL	50
APPENDIX 6. SAMPLE CALCULATION FOR TRIGLYCERIDE	
STRUCTURES OF OIL	52
APPENDIX 7. SAMPLE CALCULATION FOR ALIGNMENT STRUCTURES OF	
TRIGLYCERIDE MOLECULES IN REFINED OIL	56
APPENDIX 8. COMPUTED VALUES FOR ALIGNMENT STRUCTURES OF	
TRIGLYCERIDE MOLECULES IN REFINED OIL	58
VITA	60

LIST OF TABLES

TABLE	PAGE
1. Characteristics of Pumpkin Seed Oil	11
2. Composition of Fatty Acids in Pumpkin Seed Oil.	13
3. Iodine Values and Percentage Pumpkin Seed Oil Liquid at Low Temperatures.	24
4. Weight Percentage of Fatty Acids in Each Fraction and Refined Oil Glycerides.	27
5. Fatty Acid Composition (Mole %) of Different Triglyceride Fractions of Naked-Seeded Pumpkin Seed Oil and the Corresponding 2-Monoglycerides.	28
6. Triglyceride Composition of <u>Cucurbita pepo</u> (var. <u>Lady Godiva</u>) Pumpkin Seed Oil and Some Vegetable Oils.	30
7. Alignment Structures of Triglyceride Molecules of Naked- Seeded Pumpkin Seed Oil and Its Fractions	31

LIST OF FIGURES

FIGURE	PAGE
1. Flow Chart for Cold Fractionation of Pumpkin Seed Oil. . . .	25

CHAPTER I

INTRODUCTION

The pumpkin belongs to the Cucurbitaceae family, which includes Cucurbita maxima, C. pepo, C. moschata, C. ficifolia, and C. turbaniformis. The first two species are cultivated in Europe. Pumpkins are usually grown in parts of Europe, Asia, Africa, South America, China, and North America. C. pepo is cultivated in numerous varieties which include some of the common pumpkins and squashes. The fruit varies considerably in size, shape, and color and contains numerous seeds which usually are between 1.1 and 2.6 cm long, 0.9 to 1.5 cm wide, and 4.2 mm thick. The kernels constitute about three-fourths of the weight of the seed. Oil content of the seed is usually between 35 and 38% of the weight of the whole seed or about 48% of the weight of the kernel. Of the major oil seeds, the naked-seeded pumpkin seed contains 39.1% oil, while soybeans, cottonseed, and shelled peanuts contain 17, 23, and 48%, respectively. Robinson (65) reported the seeds of the variety Lady Godiva (C. pepo) pumpkin seed contained 37% oil.

When the external seed coat of the naked-seeded pumpkin seed is removed, the white cotyledons which contribute about 90% of the seed weight are exposed.

Hexane extract of the ground whole seeds is dark green in color, but the color of the oil can be bleached to a light yellow. The percentages of individual fatty acids in the oil reported recently are palmitic, 11.2; stearic, 5.1; oleic, 28.53; and linoleic, 55.2 (52). The oil according to Bastin and Bastin contains at least 14 different

sterols. The content of free fatty acids is 2.07% oleic (52). C. pepo is reportedly less susceptible to deterioration, which certainly is reflected in the quality of the obtained oil (82).

The possibility exists that the Lady Godiva cultivar of the naked-seeded pumpkin released in 1972 by the United States Department of Agriculture could produce yields which are sufficient to warrant growing the crop for production of seeds as a commercial oil source. Nothing has been reported concerning the triglyceride composition of the oil or the crystal formation of the oil when fully hydrogenated. Triglyceride composition determines largely crystal size of a fully hydrogenated oil which in turn determines texture of a plastic fat. Such information is needed for the oil to become a commercial oil source in the United States, since many food preparations require a plastic fat with a smooth texture.



CHAPTER II

LITERATURE REVIEW

Brief History of TG Structure Determination

Fifty years ago the study of the structure of TG molecules was begun by Hilditch (27). This study was followed some 25 years later by Kartha (33-37) who verified experimentally some of the more recent theoretical aspects of the problem. The publications of Hilditch (27-29) and Kartha (35-37) are of great importance in the study of TG structures. Later methods of analysis of TG structures were reported by Dutton et al. (18,19), Scholfield et al. (68-72), Hammond and Jones (26), Jones and Hammond (32), Privett and Blank (60) and Christie and Hunter (14). The studies of Savary et al. (66), Matson and Volpenheim (53), Coleman (12) and Youngs (83,84) using the stereospecificity of pancreatic lipase hydrolysis contributed further to a more authentic comprehension of fatty acids in TG molecules. Methodology applied to the study of TG structure research was developed by Vander Wal (77,78,79) and Gustone (24). Detailed outlines of knowledge concerning the structures of TG molecules have been published by Hilditch (28), Coleman (13) and Vander Wal (80).

The interpretation of the structure of triglyceride molecules remains at a level of so-called alignment structures of TG molecules (ASTM). In ASTM two types of fatty acids--saturated (S) and unsaturated (U)--are used to express six possible molecular types of triglycerides--SSS, SUS, UUS, SSU, USU, and UUU. Data for the calculation of ASTM are based on the presumption of 1,3-random and 2-random distribution theory proposed by Vander Wal (78) and Coleman (12). This theory states: the

acyl groups occupying the C-2 hydroxyl of the glycerol moiety are distributed at random; the 1,3 positions of the glycerol moiety are identical and are occupied by identical kinds and positions of fatty acids distributed within each position at random. Perkins (58), who reported computer methods for calculation of TG structure, indicated that of all hypotheses proposed for calculation of TG structure the 1,3-random, 2-random hypotheses best approximate values for TG structure which have been obtained experimentally by other investigators. Other researchers (40,41) have reported computer methods for determination of all individual TG structures in fats and oils by this theory.

Methods Used to Determine Triglyceride Structure

Analysis of TG structure has included separation of the fat or oil into fractions containing fewer TG types than the original mixture. Fractional crystallization of oil from an acetone solution at temperatures as low as -70°C with further separation at increasing temperatures was one of the first methods utilized (27). It is assumed in this method that each final fraction contains no more than two contiguous members of four possible glyceride types, SSS, SSU, SUU, and UUU. The method of fractional crystallization may be followed with other methods to gain further insight into TG structure and composition. Other methods also have been used, such as fractional crystallization after permanganate oxidation of all unsaturated fatty acids on TG molecule (35), countercurrent distribution in which the oil is separated into numerous fractions by repeated partitioning between two immiscible liquid phases (18,19,68), and thermal gradient fractionation in which separation of fat or oil components is brought about by a temperature

gradient in the column and partitioning between two solvents (2).

Chromatographic Methods

While some of the methods cited previously are capable of isolating single TG types, chromatographic methods (paper, column, thin layer, and gas-liquid) appear to be the best means of separation of TGs and TGs from mono- and diglycerides. For very small amounts of glycerides, paper chromatography has been proved very useful for semiquantitative work (16,51).

Quantitative results have been attained when column chromatographic methods were used. Displacement chromatographic methods (25) have been applied to model mixtures of glycerides. Good results also have been reported when a reverse phase partition chromatographic procedure was used (65).

A particularly good separation of mono-, di-, and triglycerides is the silicic acid chromatographic procedure reported by Borgstrom (7). The method was modified by Quinlin and Weiser (63) and offered several advantages over that of Borgstrom (7). When silica gel rather than silicic acid was used, larger amounts of mixed glycerides could be separated with small volumes of solvent. In addition, alcohol was eliminated from all the solvent mixtures because its presence tended to cause isomerization of the monoglycerides. A silica gel column chromatographic method for the separation of mono-, di-, and triglycerides and mineral oils also has been published (64).

Thin Layer Chromatography (TLC)

The speed and versatility of thin layer chromatography (TLC) and its ability to resolve compounds with very minor differences in chemical

structure make it valuable as an analytical technique for the quantitative analysis of lipids (62). Methods for quantitative analysis by TLC may be divided into two broad groups--those which involve recovery of the separated compounds from the chromatoplate and methods based on a direct analysis of the spots on the chromatoplate (62).

Silicic acid impregnated with silver nitrate has been used widely for the analysis of TGs after the method of de Vries (17). The use of silver nitrate in an adsorbant for TLC fractionation was first demonstrated by Morris (57). Barret et al. (3) expanded the TLC technique to the separation of TGs and developed a method for the determination of TGs based on the densitometry of charred spots similar to that employed by Privett and Blank (61).

The analysis of TGs by a combination of analytical techniques in conjunction with fractionation by silicic acid impregnated with silver nitrate has been described by a number of investigators (38,49,76). Various aspects of the analysis of TGs by multiple procedures, which include fractionation with silicic acid impregnated with silver nitrate, have been reported to Kaufman and Wessel (39), Litchfield and Reiser (50) and McCarthy and Kuksis (55). A procedure for quantitative analysis of component TGs by thin layer chromatography with silver-nitrate-silicic-acid-coated plates in combination with lipase hydrolysis was carried out by Blank et al. (6). A method of argentation TLC for separating natural TGs according to their degree of unsaturation was introduced by Barret et al. (3,4). Several modifications in the method of Barret et al. (4) with respect to preparation of the chromatoplates, development and especially charring zones have been elaborated by Chobanov and Chobanova (11).

Gas Liquid Chromatography (GLC)

Regardless of whether GLC is utilized to separate and collect single TG types or to quantitatively estimate the quantity of TG types in fat, it is utilized to determine the fatty acid composition of any glyceride (48). The feasibility of GLC for TG analysis has been demonstrated in the past, and much of the initial work has been reviewed by Huebner (30). Preparative systems have been developed which have permitted the recovery of many of the peaks (42). During the last few years, GLC has been applied to the fractionation of the TG mixtures of vegetable oils (43), butter oils (44), their molecular distillates (45, 54), adulteration mixtures (46), and their chemical (47) and enzymatic (56) reconstitution products. Essentially identical systems have permitted a direct analysis of naturally occurring neutral lipid mixtures (45). Some available techniques for GLC of mono-, di-, and triglycerides and their chemical modification products were reviewed by Kuksis (48). The effect of some common gas chromatographic variables upon the resolution and recovery of the synthetic and natural TG mixtures, such as liquid phase, column length, temperatures, and other conditions, was described by Kuksis (48). The problems of quantification in TG analysis by GLC encountered with a thermal conductivity detector has been discussed by Huebner (30), and some of the same problems are also encountered with a hydrogen flame ionization detector.

Direct Methods for Determining TG Structure

Any method which can separate quantitatively single types of a fat or oil and identify the TG structure is a direct method. Several methods have been cited previously for quantitative separation of TGs.

Identification of TG structure may be simply matching R_f of the unknown TG with R_f of the known TG as in TLC methods (4), or it may be taking the isolated TG types and analyzing the fatty acid composition in each of the three positions of TG (13). Any direct method is time consuming and/or requires expensive equipment to complete the analysis.

Indirect Methods for Determining TG Structure

Any of several proposed mathematical distribution methods for the determination of TG composition may be called an indirect method. There are generally five such methods (9,58). The completely random distribution method where fatty acids are esterified randomly over all glycerol hydroxyl groups has proved to be generally incorrect (9). This method involves fatty acid analysis of oil fractions containing possibly 2-3 TG types without any question as to positional analysis of fatty acids on the molecule. The most widely accepted mathematical distribution method to date is the 1,3-random, 2-random theory (9,59,78). In this latter method the fatty acid composition of the oil TGs is determined, and the TGs are hydrolyzed by pancreatic lipase to produce a 2-monoglyceride. The fatty acid composition of this monoglyceride is determined. The fatty acids are grouped into one of three groups depending upon the oil, and the TG structures are calculated (12). The calculations may be done by hand (12,78) or by computer (40,41,58). The 1,3-random, 2-random theory method has been shown to be applicable only if a fat has been synthesized in one tissue under similar circumstances (9), and in other circumstances, a 1-random, 2-random, 3-random theory has been proposed (9). However, it has been shown that for vegetable oils the 1,3-random, 2-random theory best approximates the

value for TG structure obtained by direct methods (58). Also, it has been shown from TG structure of corn oil determined by the 1-random, 2-random, 3-random theory method that the distribution of major fatty acids between positions 1 and 3 of the glycerol moiety are nearly random (8). This fact indicates the much shorter TG analysis by the 1,3-random, 2-random theory method should be adequate for determination of vegetable oil TG structure.

Analyses of TG structure of plant oils (74,75) have combined recently low temperature fractionation methods of Hilditch (28) with the 1,3-random, 2-random theory method of Coleman (12). This combination of methods has been used to check the accuracy of the 1,3-random, 2-random theory method.

TG Composition of Some Fats

The TG composition of Phulwara butter was determined by the technique of low temperature segregation by Bushell and Hilditch (10). According to these authors, the major glycerides were OPP (62%), POO (23%), PPP (8%), and OPS (7%), where P = palmitic, S = stearic, O = oleic. The content of trisaturated glycerides also was found to be more abundant than is usual in the seed fats with the observed proportions of saturated and unsaturated acids (29) in Phulwara butter.

Fatty acid and TG composition of Butea monosperma seed oil was determined by Sengupta and Basu (75) by using a combination of the technique of systematic crystallization at low temperatures, pancreatic lipase hydrolysis, and gas chromatography. The percentages of individual fatty acids were: myristic, 0.2; palmitic (P), 19.3; stearic (St), 7.4; arachidic, 1.8; behenic (B), 14.0; lignoceric, 6.2; oleic (O), 21.8;

linoleic (L), 27.8; and linolenic, 1.7. B. monosperma seed oil was constituted of SSS 3.8, SSU 3.9, SUS 40.9, USU 0.9, SUU 40.4, and UUU 10.1%, where S = saturated and U = unsaturated fatty acid. Chief component glycerides were: PLL 5.8, PLB 5.2, POL 4.8, POB 4.2, PLP 4.0, PLO 3.8, BLL 3.6, POP 3.3, PLST 3.2, and POO 3.0%. B. monosperma seed oil, on segregation by low temperature crystallization (Fraction A to I) yielded two major fractions, each representing 30% of the total. One of them (Fraction H) was richer in the content of SSS and SUS, while the other (Fraction F) was richer in UUU and SUU.

Fatty acid and TG compositions of Madhuca butyraceae seed fat were determined by Sengupta and Choudhury (74) using the same methods as mentioned before (75). The percentages of individual fatty acids were: palmitic (P), 55.6; stearic (St), 5.2; oleic (O), 35.9; and linoleic (L), 3.3. The special characteristic of the seed fat was its content of POP 52.5%, PLP 4.9%, POSt 8.6%, POO 14.4%, and PPP 7.7%. The 2-monoglycerides obtained by lipolysis of this fat and its least soluble fraction contained 13.0% and 29.3% saturated acids, respectively.

Pumpkin Seed Oil Characteristics

The characteristics of pumpkin seed oil, based on the available literature, are presented in Table 1 (20,23,59,73).

Eighteen samples of pumpkin seed oil were examined also by Markovic and Bastic (52), and the following indices were determined: specific gravity, 0.920-0.921; refractive index, 1.472-1.474; saponification number, 185.5-195.3; iodine value, 121-126; unsaponifiable matter, 0.5-0.73; and free fatty acids, 2.07%.

Table 1. Characteristics of pumpkin seed oil.^a

Characteristics	Sample			
	(6) ^b	(6)	(1)	(8)
Specific gravity (20°C)	0.9159	0.9179	0.903-0.909 ^{ta}	-----
Refractive index (20°C)	1.4737	1.4714	1.466-1.469 ^a	1.4741
Saponification number	174-196.5	189.4-191.5	185-198	190.8
Iodine value	116-120	121-132.6	117-130	116.4
Unsaponifiable matter	0.6-1.8	0.5-1.06	0.6-1.5	0.8
Acid number	4-12	0.5	-----	0.7

^aFrom the literature (20,23,59,73).^bSample numbers.

Markovic and Bastic (52) determined the fatty acid composition of the oil by GLC which is shown in Table 2.

No reports were found in the literature concerning the TG structure or TG composition of the oil from the naked-seeded pumpkin seed.

Table 2. Composition of fatty acids in pumpkin seed oil.^a

Fatty Acid	Sample		
	A	B	C
	$\bar{x}$		
Palmitic	11.2	11.2	11.2
Stearic	5.0	5.3	5.0
Oleic	27.1	28.1	30.4
Linoleic	56.7	55.5	53.4

^aFrom the literature (52).

CHAPTER III

MATERIAL AND METHODS

Source of Naked-Seeded Pumpkin Seeds

Naked-seeded pumpkin seeds were planted during the spring of 1978 at The University of Tennessee Plant Science Farm, Knoxville, Tennessee. Mature pumpkins were harvested in the fall of 1978, and the seeds were removed by hand. Pumpkin seeds were dried in a forced-air drier at 35°C for 16 hours and stored in a closed plastic bag at -15°C until ground.

Treatment of Naked-Seeded Pumpkin Seeds after Storage

Portions of pumpkin seeds were subjected to the following procedures.

Extraction of Oil

Whole, air-dried seeds were passed through a 30 mesh screen in a hammer mill and oil was extracted by hexane for 16 hours in a large Soxhlet apparatus. Solvent was removed from the extract by rotary evaporation at 35°C-45°C leaving a dark green crude oil.

Bleaching (81)

Crude oil was weighed into a stainless steel beaker, and nitrogen gas was introduced to the surface of the oil. Simultaneously, a stirrer in the oil was started, and the oil was heated to 70°C. Once the oil reached 70°C, heating and stirring were discontinued, and the weighed

bleaching agent¹ (Appendix 1) was added quickly. The oil was shaken gently until the powder was dispersed while the blanket of nitrogen gas was maintained over the oil surface. The oil was heated to 110°C in approximately 5 minutes and stirred at this temperature for 30 minutes. Heating was discontinued; the oil was cooled to 90°C; then the stirring was stopped. The oil was removed from the stainless steel beaker and filtered with suction through Whatman #1 filter paper in a Buchner funnel. Filtration was repeated four times.

Alkali Refining

The bleached oil was treated with alkali to remove the free fatty acids (FFA) in the oil (81).

A solution of approximately 0.1N sodium hydroxide (NaOH) was prepared by dissolving an appropriate quantity of NaOH in deionized water which had been boiled for 10 minutes to remove carbon dioxide. The solution was then cooled. The NaOH was standardized against potassium acid phthalate (Appendix 2, number 1). The percentage of FFA in the oil was determined by the AOCS official method Ca 5a-40 (1). In this procedure, 50 ml of hot, neutralized 95% ethyl alcohol was added to 28.20 g of oil, and the oil was titrated with a 0.103N NaOH to a pink endpoint using phenolphthalein indicator. The FFA content in the oil was 1.15% (Appendix 2, number 2). Next, 500 g of refined oil was weighed into a 1000 ml beaker and stirred during heating to 95°C. A sufficient amount of 8N NaOH in 10% NaCl for neutralizing the FFA plus 10% excess (28.04 ml,

¹A mixture of 0.25% (based on oil weight) activated carbon and 2.00% (based on oil weight) natural earth which was obtained from Swift's Edible Oil, Chattanooga, Tennessee.

Appendix 2, number 3) was dropped into oil at 100 drops/minute with stirring. The neutralized oil was poured into a 500 ml separatory funnel and allowed to stand 30 minutes. A brown-colored liquid layer formed and was discarded. The oil was washed with 10 ml of 0.1N NaOH. Washing with dilute alkali was repeated five times. After alkali washing, the oil was washed with hot water (95°C) 10 times.

Deodorization

The bleached, alkali-refined oil was subjected to deodorization as follows.

A burette was filled with boiled distilled water. Pieces of filter paper were placed into a 250 ml distillation flask for quite evaporation of water. A second distillation flask was filled with oil to three-quarters capacity. A steam inlet tube was inserted as low as possible into the flask containing the oil. A cold trap, filled with dry ice, was connected to a manometer and vacuum. During the process, a measured amount of water was allowed to flow from the burette into the distillation flask containing filter paper, and the water was heated to form steam. The desired vacuum (124.5 Torr) was established, and the oil in the deodorizer distillation flask was heated and maintained at approximately 140°C. Steam was introduced into the hot oil, the oil was deodorized by steam distillation for 60 minutes, and the oil was cooled to 60°C. The oil in the deodorizer was maintained in a closed system under vacuum until fresh air was carefully admitted from outside the laboratory to avoid the contamination by laboratory odors.

Quantitative Fractional Crystallization at Low Temperatures

Refined oil (50 g) was segregated according to Hilditch and Williams (29) into four fractions with varying degrees of unsaturation by stepwise crystallization at low temperatures. The oil was crystallized from 5 volumes of acetone at -30°C to 0°C , increasing the temperature by 10°C in each successive stage.

Acetone was distilled over anhydrous calcium chloride to prepare anhydrous acetone. Two hundred fifty milliliters of anhydrous acetone were mixed with 50 g of refined oil, and the solution was kept at -30°C for 5 days. At the end of 5 days, the liquid oil and acetone were separated from the solid fat. The acetone was removed from the oil by rotary evaporation at 30°C – 45°C . The oil which remained liquid at -30°C was called fraction A. The oil which crystallized at -30°C was dissolved in 5 volumes of acetone and was stored at -20°C for 5 days. Again, the oil and acetone were separated from the solid, and the acetone was removed as formerly described. The oil which remained liquid at -20°C was called fraction B. The oil which crystallized at -20°C was dissolved in 5 volumes of acetone and stored at -10°C for 5 days. Again, the oil which was liquid after storage was separated from the solid fat. The oil remaining as liquid at -10°C was called fraction C. The fat which was solid at -10°C was dissolved in 5 volumes of acetone and stored at 0°C for 5 days. The liquid oil at 0°C represented almost 95% of the remaining oil, and no further fractionation was made. The liquid oil at 0°C plus the solid at 0°C were combined and called fraction D. All fractions were stored at 4°C under nitrogen for further analyses.

Separation of TGs

The TG portion of refined oil and each fraction were separated by a column chromatographic method as follows (31): Silica gel (Research Specialties Company, 200 South Garrad Boulevard, Richmond, California) was dried in the oven at 200°C for 2 hours in a sealable container. After drying, the gel was allowed to stand in a desiccator at room temperature for 30 minutes. Dried silica gel was weighed into a 400 ml beaker, 5% distilled water (w/w) was added, and thoroughly mixed. Then, 30 g of hydrated silica gel were weighed into a 250 ml beaker. The column was filled about half full with petroleum ether, and hydrated silica gel was added to the column with tapping to dispel air bubbles and to insure uniform packing of the column.

When the level of the petroleum ether fell to 2 cm above the top of the silica gel, 5.0 ± 0.01 g of oil were added to the column in 20 ml of benzene. The eluate was collected in a 400 ml beaker. When the benzene level fell to 2 cm above the silica gel, the sample beaker was rinsed with 10 ml of benzene, which was added to the column. This washing was repeated two times more. A 250 ml separatory funnel was connected to the column, and 250 ml of benzene were allowed to flow through the column. The benzene fraction which contained only the TGs (not the mono- or diglycerides) was collected. Most of the benzene was removed on a steam bath under a hood. The final benzene vapors were removed from the TG portion by heating in a vacuum oven at 70°C until a constant weight was obtained. TGs were stored at 4°C until preparation of methyl esters.

Preparation of 2-Monoglycerides

Pancreatic Lipase Hydrolysis

TGs of refined oil and each segregated fraction were subjected to selective enzymatic hydrolysis by pancreatic lipase to prepare 2-monoglycerides as suggested by Coleman (12). Two grams of TGs were dispersed in 20 ml of a 1.2M $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ buffer, pH 8.5. Four milliliters of 22% (w/w) $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ solution, 0.2 ml of 25% (w/v) bile salt solution and 100 mg pancreatic lipase (Activity: 110-220 units/mg protein, where one unit hydrolysis equals 1.0 microequivalent of fatty acid from a TG in 1 hour at 37°C, at the indicated pH and incubation time; Lot 66C-0379; No. 3126 lipase; E.C. No. 3.1.1.3; Type II, Sigma Chemical Co., St. Louis, Missouri) were added. Hydrolysis was carried out at 37.5°C, and the pH was maintained at 8.5 throughout by addition of NH_4OH . When two-thirds of the fatty acids had been liberated (reaction time was 42.5 minutes), the pH was brought to 1.0 by the addition of 4N HCl, and the mixture was extracted with five 30 ml portions of ethyl ether. The ether was removed from the extract by a rotary evaporator at 45°C and the total volume reduced to 3-5 ml.

Separation and Isolation

The 2-monoglycerides were separated from the concentrated ether extracts by TLC. The concentrated ether extracts and a standard (2-monopalmitin) were applied to a 20 x 20 cm preparative thin layer silica gel plate (1000 μ thick, Whatman PKL5), and the plate was developed in n-hexane:ethyl ether:acetic acid (80:20:0.25 v/v/v). The separated monoglyceride was visualized by spraying the plate with 2-dichlorofluorescein. The 2-monoglyceride was located on the TLC

plate under ultraviolet light as the spot having the same R_f value as a standard. The 2-monoglyceride and silica gel were scraped off the plate, and the 2-monoglyceride was eluted with ethyl ether from the silica gel through a fine porosity glass fritted filter. The solvent was removed from the 2-monoglyceride by means of rotary evaporation at 35°C.

Fatty Acid Determination of TGs and 2-Monoglycerides

Fatty acid composition (weight percent of each acid) of refined oil and fractions A, B, C, and D for both the TG and 2-monoglycerides was determined after the glycerides had been converted to fatty acid methyl esters (A.O.C.S., 1). The methyl esters of the fatty acids (C_{16} - C_{20}) were separated on a 6 feet-by-1/4-inch O.D. stainless steel column packed with 10% EGSX on 60/80 chromosorb W/AW (Applied Science Laboratories). The column was in a model 2600 Gas Chromatograph equipped with dual flame ionization detectors and Shimadzu Model Chromotopac E-1 electronic integrator-computer. The conditions of the GLC were: nitrogen flow rate, 40 ml/min; hydrogen flow rate, 30 ml/min; air flow rate, 300 ml/min; injection temperature, 225°C; detector temperature, 225°C; and column temperature, 170°C. Standards of methyl esters of fatty acids that contained known percentages of the fatty acids, C_{16} to C_{20} , including $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{20:0}$, and $C_{18:3}$ were obtained from Applied Science Laboratories, State College, Pennsylvania, and used to check accuracy and precision of the GLC procedure.

Calculation of Iodine Value

The iodine value of the refined oil and each fraction was calculated from the fatty acid composition of the respective TG (Appendix 3).

Calculation of TG Structure

The TG structure of refined oil was calculated according to Coleman (12). Since the oil contained approximately 50% oleic, 30% linoleic, and the remainder composed of mainly palmitic and stearic, the fatty acid composition was grouped into saturated (S, palmitic, stearic, and arachidic), oleic (O), and unsaturated (U, linoleic and linolenic acids).

The mole weight percentage of each acid in each TG and 2-monoglyceride was calculated as shown in Appendix 4. Sample calculations for the TG structure of the refined oil are also shown in the Appendix 6. The TG structure of each fraction was calculated in the same manner.

After the TG structure was determined, the alignment structure of TG molecules where only two types of fatty acids, saturated (S) and unsaturated (U), are expressed was determined (40). The TGs containing only unsaturated fatty acids (OOO, UUU, OOU, UOU, OUO, OUU) were combined to form the structure of TGs, UUU. The TGs containing two saturated fatty acids in position 1 and 2 of the glyceride and unsaturated fatty acid in position 3 (SSU and SSO) were combined to give a TG alignment, SSU. TGs containing saturated fatty acid in position 1 and 3 and unsaturated fatty acids in position 2 (SUS and SOS) were combined to give TG alignment SUS. TGs containing unsaturated fatty

acids in positions 1 and 3 and saturated fatty acids in position 2 of the glycerides (USU, OSO, and OSU) were combined to give TG alignment, USU. TGs containing unsaturated fatty acid in positions 1 and 2 and saturated in position 3 of the glycerides (SUU, SOO, SOU, and SUO) were combined to give a TG, SUU. The other structure of TGs, SSU, had only one value (Appendix 8).

CHAPTER IV

RESULTS AND DISCUSSION

Extracted Oil Properties

The naked seeds of C. pepo pumpkin are large and dark green in color compared with the common pumpkin seeds which are nearly white. The hexane extract from the ground whole seeds was also dark green in color. Crude oil bleached with activated charcoal and bleaching clay resulted in a light yellow oil. Before alkali refining, the oil had a free fatty acid (FFA) content of 1.15% expressed as oleic and after refining, 0.026%. Refined oil, when deodorized, became slightly lighter in color.

Markovic and Bastic (52) reported that raw pumpkin seed oil (Cucurbita pepo) was green in color and did not exceed 2.07% oleic as FFA content. The oil which these investigators studied was obtained from commercial centers. The oil extracted in the laboratory for the present study, therefore, was of good quality.

Oil Fractionation

Table 3 shows the calculated iodine value and percentage of oil found in each fraction after low temperature crystallization.

Figure 1 shows a flow chart of the cold fractionation of pumpkin seed oil.

Table 3. Iodine values and percentage pumpkin seed oil liquid at low temperatures.

Liquid Fraction	Temperature -----°C-----	Liquid Oil ^a ---%---	Iodine Value ^b g I/100 g oil
A	-30	8.7	99.7
B	-20	20.1	93.8
C	-10	27.9	91.4
D	-0-	43.3	80.7

^a Average of two runs.

^b Calculated from fatty acid composition of triglyceride.

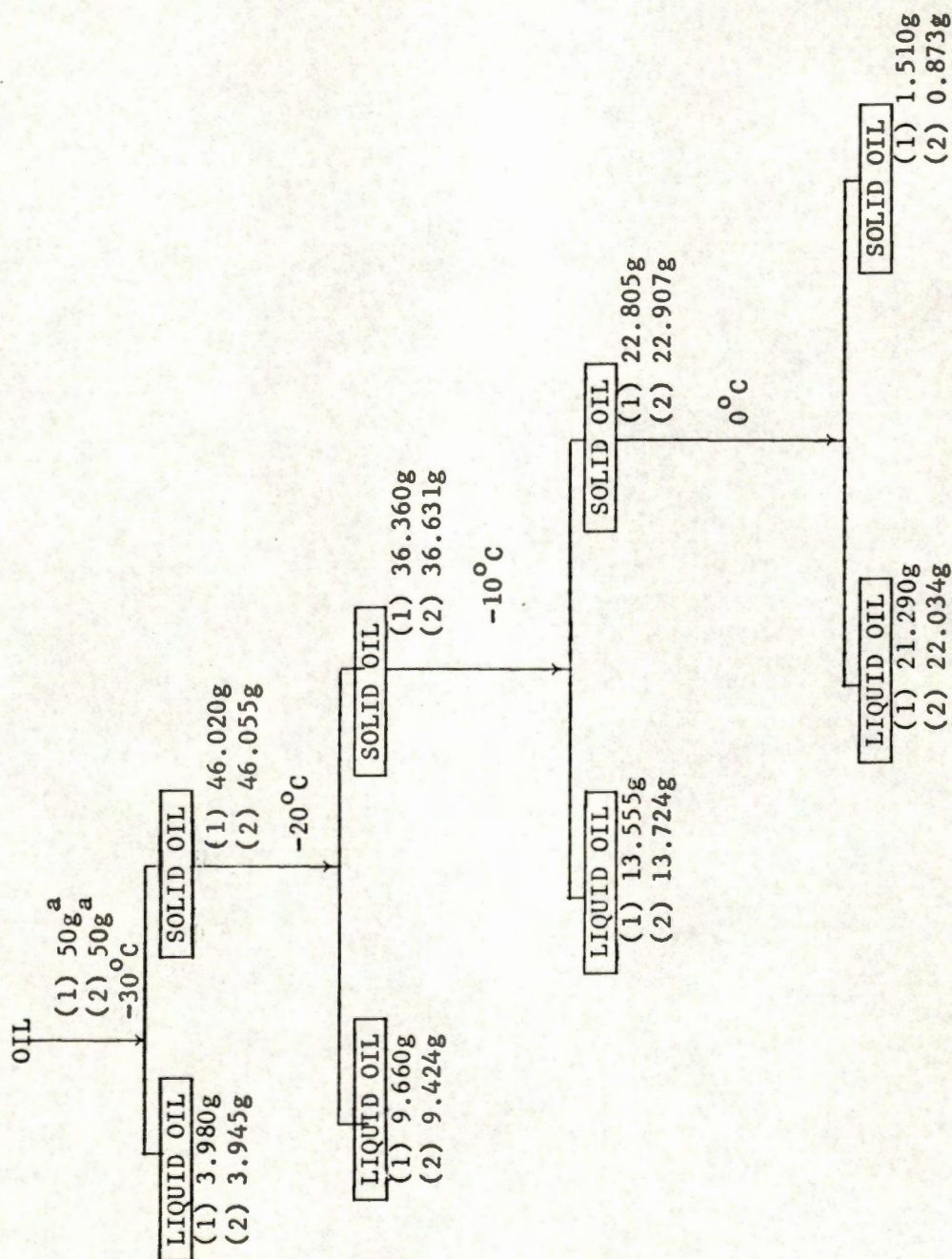


Figure 1. Flow chart for cold fractionation of pumpkin seed oil.

^aRepresents run 1 and run 2.

Fatty Acid Composition of TG and 2-Monoglyceride

The weight percentage of fatty acids in TGs and 2-monoglycerides is shown in Table 4. The fatty acid composition of the refined oil TG from pumpkin seeds grown in 1978 at The University of Tennessee Plant Science Farm, Knoxville, is comparable to the fatty acid composition of oil extracted from seeds grown at the same place during 1977 (15). However, the oleic acid (almost 50%) is from 1.2 to 2 times higher than oleic acid content in pumpkin seed oil reported by Markovic and Bastic (52). Also, the linoleic acid content is from 1.4 to 1.8 times less than that reported by these same authors. Other workers (21,22) have reported fatty acids composition of the naked-seeded pumpkin seed oil to be in agreement with the present data.

A comparison of the TG fatty acid composition with that of the monoglyceride shows that the 2-position on the pumpkin seed TG is esterified preferentially with unsaturated fatty acids, particularly linoleic (42-56% except in fraction C in monoglyceride compared with 26-36% in TG, Table 4). Other investigators (12,74,75) have found that the 2-position of vegetable or plant oil is preferentially esterified with the unsaturated fatty acids also.

Fatty acid composition of TGs and the corresponding 2-monoglycerides of all the fractions plus the refined oil was converted into mole percentage and is shown in Table 5. Calculations are shown in Appendix 4.

It is possible to calculate the percentage fatty acid in TGs and monoglycerides of the refined oil from the fatty acid composition of the same glycerides in the fraction at low temperatures. In Table 5, these

Table 4. Weight percentage of fatty acids in each fraction and refined oil glycerides.

Glyceride		Fraction ^a			
Fatty Acid	Refined Oil	A (8.7%)	B (20.1%)	C (27.9%)	D (43.3%)
		-----%			
<u>Triglyceride</u>					
C _{16:0}	13.80	9.50	11.31	12.98	17.50
C _{18:0}	4.88	2.46	3.47	4.74	7.42
C _{18:1}	49.90	50.79	52.49	50.74	48.49
C _{18:2}	31.15	36.94	32.25	32.20	26.11
C _{20:0}	---	---	---	0.076	0.28
C _{18:3}	0.27	0.30	0.28	0.27	0.19
<u>2-Monoglyceride</u>					
C _{16:0}	3.25	2.58	2.22	5.34	3.09
C _{18:0}	1.00	0.63	0.64	1.45	0.98
C _{18:1}	53.34	39.87	49.31	62.17	48.83
C _{18:2}	42.09	56.62	47.65	30.91	46.78
C _{20:0}	---	---	---	---	---
C _{18:3}	0.32	0.30	0.17	0.12	0.22

^aNumber in parentheses represents fraction of oil remaining liquid at A, -30°C; B, -20°C; C, -10°C and D, 0°C.

Table 5. Fatty acid composition (mole %) of different triglyceride fractions of naked-seeded pumpkin seed oil and the corresponding 2-monoglycerides.

Fatty Acid	Fractions ^a												Computed Values Refined Oil	
	Refined Oil		A (8.10%)		B (19.95%)		C (27.95%)		D (44.40%)					
	TG ^b	MG ^b	TG	MG	TG	MG	TG	MG	TG	MG	TG	MG	TG	MG
C16:0	14.96	3.56	10.34	2.82	12.32	2.43	14.09	5.84	18.92	3.38	15.63	3.85		
C18:0	4.77	0.98	2.41	0.62	3.40	0.63	4.63	1.43	7.23	0.97	5.38	1.01		
C18:1	49.11	53.01	50.46	39.60	51.89	49.04	49.99	61.70	47.60	48.56	49.55	51.80		
C18:2	30.87	42.11	36.76	56.65	32.10	47.73	30.95	30.90	25.81	46.86	29.57	43.55		
C20:0	---	---	---	---	---	---	0.07	---	0.25	---	0.16	---		
C18:3	0.28	0.34	0.31	0.30	0.28	0.17	0.27	0.12	0.19	0.23	0.24	0.19		

^aNumber in parentheses indicates percentage of oil remaining liquid at A, -30°C; B, -20°C; C, -10°C and D, 0°C.

^bTG = triglyceride and MG = monoglyceride.

computed values are shown. There is good agreement between the computed fatty acid composition of the refined oil and that actually measured. Calculations for computed values are shown in Appendix 5.

The fatty acids of TGs and 2-monoglycerides, when grouped into saturated, oleic, and unsaturated and the original TG composition calculated according to Coleman (12), are shown in Table 6. Calculations are shown in Appendix 6.

TG structure of olive oil and maize oil which were calculated from the same grouping of fatty acids is also shown in Table 6 for comparison. The chief glycerides of pumpkin seed oil were S00, 23.5%; 00U, 13.02%; 000, 12.01%; 0UU, 10.36%; and 0US, 10.8%. In contrast, olive oil which had a higher percentage oleic acid than pumpkin seed oil had 39.2% 000, 26.0% S00, 13.7% 00U, and a much lower percentage of 0US and 0UU than the pumpkin seed oil. Maize oil with a lower percentage of oleic acid than pumpkin seed oil had only 3.38% S00, 2.14 000, 8.04% 00U, 7.89% 0US, and 18.56% 0UU. The most abundant TG in olive oil was 000 (39.2%); the most abundant TG in maize was 0UU (18.56%) and UUU (17.75%).

The six alignment structures of the TGs of pumpkin seed oil are shown in Table 7 as well as the alignment structure of each segregated fraction of the oil. Calculations are shown in Appendix 7. It is possible to calculate the alignment structure of the oil from the alignment structure of the segregated fractions (Appendix 8). The calculated alignment structures of the oil agree well with the results determined for the oil without fractionation. This fact gives confidence that the TG structure of the naked-seeded pumpkin seed oil has been determined.

Table 6. Triglyceride composition of Cucurbita pepo (var. Lady Godiva) pumpkin seed oil and some vegetable oils.

Triglycerides	Pumpkin ^a	Olive ^b	Maize ^c
SOS	3.58	4.3	1.31
SOO	13.31	26.0	3.38
SOU	7.16	4.5	6.25
OOO	12.01	39.2	2.14
OOU	13.32	13.7	8.04
UOU	3.58	--	7.56
SSS	0.28	0.1	--
SSO	1.06	0.2	--
SSU	0.57	--	--
OSO	0.98	0.3	--
OSU	1.06	0.1	--
USU	0.28	--	--
SUS	2.84	0.5	3.05
UUS	5.69	0.5	15.36
OUS	10.58	3.0	7.89
OUO	9.84	4.6	4.99
OUU	10.59	1.6	18.56
UUU	2.85	0.2	17.75

^aPresent results.

^bColeman's results (12).

^cKoman's results (41).

Table 7. Alignment structures of triglyceride molecules of naked-seeded pumpkin seed oil and its fractions.

Samples	Oil --%--	Glycerides					
		SSS	SSU	SUS	USU	SUU	UUU
-----mole %-----							
Refined oil	100.00	0.285	1.629	6.423	2.323	36.752	52.575
Fraction A	8.10	0.086	0.878	2.581	2.248	26.450	67.188
Fraction B	19.95	0.123	0.938	4.176	1.785	31.808	60.751
Fraction C	27.95	0.368	2.428	5.057	3.997	33.304	54.837
Fraction D	44.40	0.520	1.871	12.256	1.681	44.062	39.601
Computed value (A-D) refined oil	100.00	0.365	1.764	7.880	2.394	37.233	50.229

CHAPTER V

SUMMARY

This study was designed to determine the TG structure and fatty acid composition of oil extracted from the naked-seeded pumpkin seeds (Cucurbita pepo var. Lady Godiva). Fatty acid and TG composition have been determined by a combination of the techniques of systematic crystallization at low temperatures, pancreatic lipase hydrolysis, thin layer chromatography, and gas liquid chromatography of methyl esters of fatty acids.

The TG portion of the refined oil was separated and then hydrolyzed by pancreatic lipase, and the 2-monoglyceride was isolated. The fatty acid composition of TGs and corresponding 2-monoglycerides were determined by GLC analysis of the methyl esters of the fatty acids. The TG composition was confirmed by quantitative fractional crystallization of the refined oil, determination of TG composition of each fraction, and calculation of the original TGs from the TGs of each fraction.

Refined oil was quantitatively segregated into four fractions (A, 8.10%; B, 19.95%; C, 27.95%; D, 44.40%) with varying degrees of unsaturation (calculated iodine values: 99.68, 93.82, 91.43, 80.72, respectively) by stepwise crystallization from 5 volumes of acetone at -30°C to 0°C in each successive stage.

The results of GLC analyses gave the following percentage of fatty acids in refined oil: palmitic, 14.96; stearic, 4.8; oleic, 49.11; and linoleic, 30.87. The TGs identified from the refined oil were: SSS, 0.28%; SSU, 1.63%; SUS, 6.42%, USU, 2.32%; SUU, 36.75%; and UUU, 52.58; where S = saturated fatty acid and U = unsaturated fatty acid.

LIST OF REFERENCES

LIST OF REFERENCES

1. A.O.C.S. 1977. Official and tentative methods. 3rd ed. Chicago: Amer. Oil Chem. Soc.
2. Baker, C. A. and Williams, R. J. P. 1956. Chromatographic procedure and its application to high polymers. J. Chem. Soc. (London), p. 2352.
3. Barrett, C. B., Dallas, M. S. and Padley, F. B. 1962. The separation of glycerides by thin layer chromatography on silica impregnated with silver nitrate. Chem. Ind. (London), p. 1050.
4. Barret, C. B., Dallas, M. S. and Padley, F. B. 1963. The quantitative analysis of TG mixtures by thin layer chromatography on silica impregnated with silver nitrate. J. Amer. Oil Chem. Soc. 40:580.
5. Bastic, M., Bastic, L. J., Jovanovic, A. and Spiteller, G. 1977. Sterols in pumpkin seed oil. J. Amer. Oil Chem. Soc. 54:525.
6. Blank, M. L., Verdino, B. and Privett, O. S. 1965. Determination of triglyceride structure via silver nitrate--TLC. J. Amer. Oil Chem. Soc. 42:87.
7. Borgstrom, D. 1954. Investigation on lipid separation methods. 3. Separation of tri-, di-, and 2-monoglycerides. Acta Physiol. Scand. 30:231.
8. Brockerhoff, H. 1965. A stereospecific analysis of triglycerides. J. Lipid. Res. 6:10.
9. Brockerhoff, H. 1971. Stereospecific analysis of triglycerides. Lipids 6:942.
10. Bushell, W. J. and Hilditch, T. P. 1938. Seed fat of Madhuca butyraceae (Phulwara butter) IV. J. Soc. Chem. Ind. 57:48.
11. Chobanov, D., Tarandjiska, R. and Chobanova, R. 1976. Rapid densitometric determination of triglyceride groups by argentation thin layer chromatography. J. Amer. Oil Chem. Soc. 53:48.
12. Coleman, M. H. 1961. Further studies on the pancreatic hydrolysis of some natural fats. J. Amer. Oil Chem. Soc. 38:685.
13. Coleman, M. H. 1963. Advances in lipid research. Vol. 1. New York and London: Academic Press.
14. Christie, W. W. and Hunter, M. L. 1973. Stereospecific analysis of triglycerides that contain isotopically labeled fatty acids. Biochem. Biophys. Acta. 316:282.

15. Cunningham, S. D., Jaynes, H. O. and Melton, S. L. 1978. Chemical composition of the seeds of naked seeded pumpkin. Unpublished work.
16. Dieckert, J. W. and Reiser, R. 1956. A paper chromatographic procedure for separating 1-mono-, 1,3-di-, and triglycerides, cholesterol, and cholesteryl esters. J. Amer. Oil Chem. Soc. 33:123.
17. de Vries, B. 1962. Paper presented to the VIth I.S.F. Congress, London. Chem. & Ind., p. 1049.
18. Dutton, H. J. and Cannon, J. A. 1956. Glyceride structure of vegetable oils by countercurrent distribution. 1. Linseed oil. J. Amer. Oil Chem. Soc. 33:46.
19. Dutton, H. J., Scholfield, C. R. and Mounts, T. A. 1961. Glyceride structure of vegetable oils by countercurrent distribution. V. Comparison of natural, interesterified, and synthetic cocoa butter. J. Amer. Oil Chem. Soc. 38:96.
20. Ecky, E. W. 1954. Vegetable Fats and Oils. New York: Reinhold Publishing Company.
21. El-Gharbow, M. I. 1977. Some physical and chemical characteristics of naked pumpkin seed oil (Cucurbita pepo). Presented at the 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, Pennsylvania.
22. El-Gharbow, M. I. 1978. Fatty acid composition of naked seeded pumpkin seed oil (Cucurbita pepo) and amino acid profile of cake proteins. Presented at the 38th Annual Meeting of the Institute of Food Technologists, Dallas, Texas.
23. Grun, A. 1929. Analyse der Fette und Wachse. Springer Verlag., Berlin, Germany, p. 164.
24. Gustone, F. D. 1962. The distribution of fatty acids in natural glycerides of vegetable origin. Chem. and Ind., p. 1214.
25. Hamilton, J. G. and Holman, R. T. 1954. Displacement analysis of lipids. X. Model mixtures of glycerides. J. Amer. Oil Chem. Soc. 76:4107.
26. Hammond, E. G. and Jones, G. V. 1960. Calculation of restricted random glyceride distribution. J. Amer. Oil Chem. Soc. 37:376.
27. Hilditch, T. P. 1956. The Chemical Constitution of Some Natural Fats. 3rd ed. New York: John Wiley and Sons, Inc.
28. Hilditch, T. P. 1964. The chemical constitution of natural fats. 4th ed. New York: John Wiley and Sons, Inc.
29. Hilditch, T. P. and Williams, P. N. 1964. The Chemical Constitution of Natural Fats. 4th ed. Chapman and Hall, London, pp. 373; 441.

30. Huebner, V. R. 1961. The analysis of glyceride by high temperature gas-liquid partition chromatography. J. Amer. Oil Chem. Soc. 38:628.
31. Humko Products. 1975. Analytical procedure 145. Personal communication. Memphis, Tennessee.
32. Johnes, G. V. and Hammond, E. C. 1961. Analysis of the glyceride structure of cocoa butter by thermal gradient crystallization. J. Amer. Oil Chem. Soc. 38:69.
33. Kartha, A. R. S. 1949. Doctoral thesis, University of Madras, India.
34. Kartha, A. R. S. 1951. Studies on the natural fats, vol. 1. Ernahnlen, India: Author.
35. Kartha, A. R. S. 1953. The glyceride structure of natural fats. II. The rule of glyceride-type distribution of natural fats. J. Amer. Oil Chem. Soc. 30:326.
36. Kartha, A. R. S. 1954. The glyceride structure of natural fats. III. Factors governing the content of fully saturated glycerides. J. Amer. Oil Chem. Soc. 31:85.
37. Kartha, A. R. S. 1962. The glyceride structure of Magnifera indica seed fat. J. Sci. Ind. Res. 21A:577.
38. Kauffman, H. P. and Wessel, H. 1964. Thin layer chromatography in the field of fats. 14. The separation of triglycerides of adsorption and reversed phase chromatography. Fette, Seifen, Anstrichmittel 66:81.
39. Kauffman, H. P. and Wessel, H. 1964. The structure of triglycerides: theories and analytical methods. Fette, Seifen, Anstrichmittel 66:13.
40. Koman, V. and Kotuc, J. 1977. Computer determination of all individual structures of triglyceride molecules of fats and oils. J. Amer. Oil Chem. Soc. 54:95.
41. Koman, V. 1978. Computer determination of all individual structures of triglyceride molecules of fats and oils:II. J. Amer. Oil Chem. Soc. 55:629.
42. Kuksis, A. and Ludwig, J. Unpublished results. Cited by J. Amer. Oil Chem. Soc. 42:269.
43. Kuksis, A. and McCarthy, M. J. 1962. Gas-liquid chromatographic fractionation of natural triglyceride mixtures by carbon number. Can. J. Biochem. Physiol. 40:679.
44. Kuksis, A., McCarthy, M. J. and Beveridge, J. M. R. 1963. Quantitative gas liquid chromatographic analysis of butterfat triglycerides. J. Amer. Oil Chem. Soc. 40:530.

45. Kuksis, A. 1964. Direct gas chromatographic fractionation of mixed natural lipids of natural origin. *Can. J. Biochem.* 42:419.
46. Kuksis, A. and McCarthy, M. J. 1964. Triglyceride gas chromatography as a means of detecting butterfat adulteration. *J. Amer. Oil Chem. Soc.* 41:17.
47. Kuksis, A., McCarthy, M. J. and Beveridge, J. M. R. 1964. Triglyceride composition of native and rearranged butter and coconut oils. *J. Amer. Oil Chem. Soc.* 41:201.
48. Kuksis, A. 1965. Gas-liquid chromatography of glycerides. *J. Amer. Oil Chem. Soc.* 42:269.
49. Litchfield, C., Farguhar, M. and Reiser, R. 1964. Analysis of triglycerides by consecutive chromatographic techniques. I. Cuphea llavia seed fat. *J. Amer. Oil Chem. Soc.* 41:588.
50. Litchfield, C. and Reiser, R. 1964. Analysis of triglycerides by multiple chromatographic techniques. Presented at the World Fat Congress, Hamburg, Germany.
51. Mangold, H. K., Lamp, B. G. and Schlenk, H. 1955. Indicators for the paper chromatography of lipids. *J. Amer. Oil Chem. Soc.* 77:6070.
52. Markovic, V. V. and Bastic, L. V. 1976. Characteristics of pumpkin seed oil. *J. Amer. Oil Chem. Soc.* 53:42.
53. Matson, F. H. and Volpenheim, R. A. 1961. The use of pancreatic lipase for determining the distribution of fatty acids in partial and complete glycerides. *J. Lipid Res.* 2:58.
54. McCarthy, M. J., Kuksis, A. and Beveridge, J. M. R. 1962. Gas-liquid chromatographic analysis of the triglyceride composition of molecular distillates of butter oil. *Can. J. Biochem. Physiol.* 40:1693.
55. McCarthy, M. J. and Kuksis, A. 1964. Exploitation of the selectivity of various chromatographic techniques for the study of the triglycerides structure of natural fats. *J. Amer. Oil Chem. Soc.* 41:527.
56. McCarthy, M. J., Kuksis, A. and Beveridge, J. M. R. 1964. Paper presented at the 6th International Congress of Biochemistry. New York (July 26-August 1). Abstracts VII, p. 585.
57. Morris, L. J. 1962. Separation of higher fatty acid isomers and vinyllogues by thin layer chromatography. *Chem. Ind. (London)*, p. 1238.
58. Perkins, E. G. 1965. Application of computer methods to the calculation of triglyceride structure. *J. Amer. Oil Chem. Soc.* 42:1032.

59. Popov, A. and Dusev, A. 1972. Isledovanie vrhu blgarskite maznini i drugi lipidi, otdel za naucnotechniceska, ikonomiceska i patentna informacija, Sofija, Bulgaria, p. 54.
60. Privette, O. S. and Blank, M. L. 1961. A new method for the analysis of component mono-, di-, and triglycerides. *J. Lipid Res.* 2:37.
61. Privett, O. S. and Blank, M. L. 1963. A method for the structural analysis of triglycerides and lecithins. *J. Amer. Oil Chem. Soc.* 40:70.
62. Privett, O. S., Blank, M. L., Coddington, D. W. and Nickell, E. C. 1965. Lipid analysis by quantitative thin layer chromatography. *J. Amer. Oil Chem. Soc.* 42:381.
63. Quinlin, P. and Weiser, H. J., Jr. 1958. Separation and determination of mono-, di-, and triglycerides in monoglyceride concentrates. *J. Amer. Oil Chem. Soc.* 35:327.
64. Ravin, I. J., Meyer, R. J. and Hignchi, T. 1957. Chromatographic analysis of mixtures of mono-, di-, and tristearin containing mineral oil. *J. Amer. Oil Chem. Soc.* 34:261.
65. Robinson, R. G. 1975. Amino acid and elemental composition of sunflower and pumpkin seeds. *Agronomical Journal* 67:541.
66. Savary, P., Flanzy, J. and Desnuelle, P. 1957. Emploi de la lipase pancreatique pour l'etude de la structure des corps gras naturels. *Biochem. Biophys. Acta.* 24:414.
67. Savary, P. and Desnuelle, P. 1954. Separation chromatographique des monoglycerides-1 et des monoglycerides-2. *Bull. Soc. Chem. France* 78:936.
68. Scholfield, C. R. and Hicks, M. A. 1957. Glyceride structure of vegetable oils by countercurrent distribution. II. Soybean oil. *J. Amer. Oil Chem. Soc.* 34:77.
69. Scholfield, C. R. and Dutton, H. J. 1958. Glyceride structure of vegetable oils by countercurrent distribution. III. Safflower oil. *J. Amer. Oil Chem. Soc.* 35:493.
70. Scholfield, C. R. and Dutton, H. J. 1959. Glyceride structure of vegetable oils by countercurrent distribution. IV. Cocoa butter. *J. Amer. Oil Chem. Soc.* 36:325.
71. Scholfield, C. R., Nowakowska, J. and Dutton, H. J. 1961. Glyceride structure of vegetable oils by countercurrent distribution. VI. Corn oil. *J. Amer. Oil Chem. Soc.* 38:175.
72. Scholfield, C. R. 1961. Countercurrent distribution. *J. Amer. Oil Chem. Soc.* 38:562.

73. Schormuller, Y. 1969. Handbuch der Lebensmittelchemie, Band IV Fette und Lipide. Springer Verlag, Berlin, Germany, p. 53.
74. Sengupta, A. and Choudhury, S. K. R. 1978. Triglyceride composition of Madhuca butyraceae seed fat. J. Amer. Oil Chem. Soc. 55:621.
75. Sengupta, A. and Basu, S. P. 1978. The triglyceride composition of Butea monosperma seed oil. J. Amer. Oil Chem. Soc. 55:533.
76. Subbaram, M. R. and Youngs, C. G. 1964. Determination of the triglyceride structure of fats: Distribution of individual saturated and unsaturated acids. J. Amer. Oil Chem. Soc. 41:445.
77. Vander Wal, R. J. 1960. Calculation of the distribution of the saturated and unsaturated acyl groups in fats from pancreatic lipase hydrolysis data. J. Amer. Oil Chem. Soc. 37:18.
78. Vander Wal, R. J. 1963. The determination of glyceride structure. J. Amer. Oil Chem. Soc. 40:242.
79. Vander Wal, R. J. 1963. Molecular dynamics and glyceride structure. J. Amer. Oil Chem. Soc. 40:256.
80. Vander Wal, R. J. 1964. Advances in Lipid Research. Vol. 2. New York and London: Academic Press.
81. Van Rede, C. 1966. Laboratory Handbook for Oil and Fats Analysis. London and New York: Academic Press.
82. Vatta, J. B. 1958. Vpliv proizvodnih postopkov na kvaliteteo vucnega olja. Diplomski rad, Univerzitet u Ljubljani, Yugoslavia, p. 4.
83. Youngs, C. G. 1959. Glyceride structure of fats. J. Amer. Oil Chem. Soc. 36:664.
84. Youngs, C. G. 1961. Determination of the glyceride structure of fats. J. Amer. Oil Chem. Soc. 38:62.

APPENDIXES

CRANE & CREST

APPENDIX 1

BLEACHING OF OIL

GRANT & CO. RESI

Material (% Based on oil)

Weight

Crude oil

550 g

Earth--2

$550 \times 0.02 = 11 \text{ g}$

Activated carbon--0.25

$550 \times 0.0025 = 1.375 \text{ g}$

APPENDIX 2

REFINING OF OIL



(1) Normality of NaOH solution

$$\frac{\text{Wt (g) of KHC}_8\text{H}_4\text{O}_4}{\text{Vol (ml) of NaOH}} \times \frac{\text{meq}}{0.2042}$$

$$\frac{0.5003}{23.79} \times \frac{1}{0.2042}$$

0.103 N

(2) Free fatty acid (as oleic)

$$\frac{\text{ml of alkali} \times \text{N} \times 28.2}{\text{Wt of sample}}$$

$$\frac{11.2 \times 0.103 \times 28.2}{28.30 \text{ g}}$$

1.15%

where, N = normality of NaOH solution

(3) Solution of 8N NaOH such that there is 10% excess of alkali

Amount of 8N NaOH in 10% NaCl required

$$\frac{\text{Wt of oil} \times \text{FFA} \times 1.1 \times 1000}{100 \times 0.8 \times 282}$$

$$\frac{500 \times 1.15 \times 1.1 \times 1000}{100 \times 0.8 \times 282}$$

28.036 ml



APPENDIX 3

CALCULATION OF IODINE VALUES

(1) Fraction A (-30°C)

Average molecular weight =

$$256 \times 0.095 + 284 \times 0.0246 + 282 \times 0.51 + 280 \times 0.37 + 278 \times 0.003 + 41 = 320.56$$

Weight of iodine combined =

$$126.9 \times 2 \times 0.51 + 126.9 \times 4 \times 0.37 + 126.9 \times 6 \times 0.003 = 319.53$$

$$\text{Iodine value} = \frac{319.53}{320.56} \times 100 = 99.68 \text{ g I/100 g oil}$$

(2) Fraction B (-20°C)

Average molecular weight =

$$256 \times 0.11 + 284 \times 0.035 + 282 \times 0.525 + 280 \times 0.32 + 278 \times 0.003 + 41 = 317.58$$

Weight of iodine combined =

$$126.9 \times 2 \times 0.525 + 126.9 \times 4 \times 0.32 + 126.9 \times 6 \times 0.003 = 297.96$$

$$\text{Iodine value} = \frac{297.96}{317.58} \times 100 = 93.82 \text{ g I/100 g oil}$$

(3) Fraction C (-10°C)

Average molecular weight =

$$256 \times 0.13 + 284 \times 0.047 + 282 \times 0.507 + 280 \times 0.322 + 312 \times 0.0008 + 278 \times 0.0027 + 41 = 321.76$$

Weight of iodine combined =

$$126.9 \times 2 \times 0.507 + 126.9 \times 4 \times 0.322 + 126.9 \times 6 \times 0.0027 = 294.18$$

$$\text{Iodine value} = \frac{294.18}{321.76} \times 100 = 91.43 \text{ g I/100 g oil}$$

(4) Fraction D (0°C)

Average molecular weight =

$$256 \times 0.175 + 284 \times 0.074 + 282 \times 0.485 + 280 \times 0.26 + \\ 312 \times 0.0028 + 278 \times 0.0019 + 41 = 317.79$$

Weight of iodine combined =

$$126.9 \times 2 \times 0.485 + 126.9 \times 4 \times 0.26 + 126.9 \times 6 \times 0.0019 = 256.52$$

$$\text{Iodine value} = \frac{256.52}{317.79} \times 100 = 80.72 \text{ g I/100 g oil}$$



APPENDIX 4

SAMPLE CALCULATION OF MOLE PERCENTAGE OF EACH FATTY ACID



(a) Molecular weight of fatty acids

FA	Formula	MW	FA	Formula	MW
C _{16:0}	C ₁₅ H ₃₂ COO	256	C _{18:2}	C ₁₇ H ₃₂ COO	280
C _{18:0}	C ₁₇ H ₃₆ COO	284	C _{18:3}	C ₁₇ H ₃₀ COO	278
C _{18:1}	C ₁₇ H ₃₄ COO	282	C _{20:0}	C ₁₉ H ₃₀ COO	312

(b) Mole percentage of fatty acids in refined oil triglycerides

FA	No. of moles ($\frac{\text{wt \%}}{\text{MW}}$)	Mole % ($\frac{\text{No. of moles}}{\text{total \# of moles}} \times 100$)
C _{16:0}	$\frac{13.796}{256} = 0.0539$	$\frac{0.0539}{0.3602} \times 100 = 14.9639$
C _{18:0}	$\frac{4.8845}{284} = 0.0172$	$\frac{0.0172}{0.3602} \times 100 = 4.7751$
C _{18:1}	$\frac{49.897}{284} = 0.1769$	$\frac{0.1769}{0.3602} \times 100 = 49.1116$
C _{18:2}	$\frac{31.1465}{280} = 0.1112$	$\frac{0.1112}{0.3602} \times 100 = 30.8717$
C _{18:3}	$\frac{0.273}{278} = 0.001$	$\frac{0.001}{0.3602} \times 100 = 0.2776$
Total	0.3602	99.9999

APPENDIX 5

CALCULATIONS FOR COMPUTED FATTY ACID COMPOSITION
OF REFINED OIL

(1) Triglycerides

$$C_{16:0} = 10.342 \times 0.0810 + 12.316 \times 0.4440 + 14.086 \times 0.2795 + 18.919 \times 0.4440 = 15.6318$$

$$C_{18:0} = 2.409 \times 0.0810 + 3.404 \times 0.1995 + 4.635 \times 0.2795 + 7.228 \times 0.4440 = 5.3789$$

$$C_{18:1} = 50.463 \times 0.0810 + 51.894 \times 0.1995 + 49.989 \times 0.2795 + 47.6 \times 0.4440 = 49.5467$$

$$C_{18:2} = 37.76 \times 0.0810 + 32.104 \times 0.1995 + 30.954 \times 0.2795 + 25.812 \times 0.4440 = 29.5755$$

$$C_{20:0} = 0.269 \times 0.2795 + 0.193 \times 0.4440 = 0.1609$$

$$C_{18:3} = 0.306 \times 0.0810 + 0.282 \times 0.1995 + 0.269 \times 0.2795 + 0.193 \times 0.4440 = 0.2420$$

(2) Monoglycerides

$$C_{16:0} = 2.823 \times 0.0810 + 2.431 \times 0.1995 + 5.839 \times 0.2795 + 3.385 \times 0.4440 = 3.8486$$

$$C_{18:0} = 0.623 \times 0.0810 + 0.633 \times 0.1995 + 1.433 \times 0.2795 + 0.968 \times 0.4440 = 1.0071$$

$$C_{18:1} = 39.604 \times 0.0810 + 49.037 \times 0.1995 + 61.702 \times 0.2795 + 48.561 \times 0.4440 = 51.7976$$

$$C_{18:2} = 56.646 \times 0.0810 + 47.727 \times 0.1995 + 30.903 \times 0.2795 + 46.859 \times 0.4440 = 43.5526$$

$$C_{18:3} = 0.304 \times 0.0810 + 0.169 \times 0.1995 + 0.123 \times 0.2795 + 0.227 \times 0.4440 = 0.1935$$

APPENDIX 6

SAMPLE CALCULATION FOR TRIGLYCERIDE STRUCTURES OF OIL

(1) TG	18.6805	49.6910	31.4195
(2) 3TG	56.0415	149.6910	94.2585
(3) MG	4.2445	53.3400	42.4135
(4) 3TG-MG	51.7970	96.3510	51.8450
(5) $\frac{3TG-MG}{2}$	25.8985	48.1755	25.9225

MG	%	DG	%	TG	%
S	4.24	SS 4.2445 X 0.258985	1.09926	SSS	
				1.09926 X 0.258985	0.28469
				SSO	
		OS 4.2445 X 0.481755	2.04481	1.09926 X 0.481755	0.52957
				SSU	
				1.09926 X 0.259225	0.28496
		US 4.2442 X 0.259225	1.10028	OSS	0.52957
				OSO	0.98514
				OSU	0.53007
		SO 53.3400 X 0.258985	13.81426	USS	
				1.1035 X 0.258985	0.28496
				USO	
				1.1035 X 0.481755	0.53009
		SOU 13.81426 X 0.259225	3.58100	USU	
				1.1035 X 0.259225	0.27755
				SOS	
				13.81426 X 0.258985	3.57769
		SOU 13.81426 X 0.259225	3.58100	SOU	
				13.81426 X 0.481755	6.65509
				SOU	
				13.81426 X 0.259225	3.58100

MG	%	DG	%	TG	%
O	53.34	OO		OOS	
		53.3400 X 0.0481755	25.69688	25.69688 X 0.258985	6.65509
				OOO	
				25.69688 X 0.481755	12.37957
				OOU	
				25.69688 X 0.259225	6.66126
U	42.4135	UO		UOS	
		53.3400 X 0.259225	13.82706	13.82706 X 0.258985	3.58100
				UOO	
				13.82706 X 0.481755	6.66126
				UOU	
				13.82706 X 0.259225	3.58432
		SU		SUS	
		42.4135 X 0.258985	10.98446	10.98446 X 0.258985	2.84481
				SUO	
				10.98446 X 0.481755	5.29182
				SUU	
				10.98446 X 0.259225	2.84745
		OU		OUS	
		42.4135 X 0.481755	20.43292	20.43292 X 0.258985	5.29182
				OUO	
				20.43292 X 0.481755	9.84366
				OUU	
				20.43292 X 0.259225	5.29672
		UU		UUS	
		42.4135 X 0.259225	10.99464	10.99464 X 0.258985	2.84745
				Uuo	
				10.99464 X 0.481755	6.29672
				UUU	
				10.99464 X 0.259225	2.85008

$$SSS = 0.28469$$

$$SSO = SSO + OSS = 0.52957 \times 2 = 1.05915$$

$$SSU = SSU + USS = 0.28496 \times 2 = 0.56992$$

$$OSO = 0.98514$$

$$OSU = OSU + USO = 0.53007 \times 2 = 1.06014$$

$$USU = 0.27755$$

$$SOS = 3.57769$$

$$SOO = SOO + OOS = 6.65509 \times 2 = 13.31018$$

$$SOU = SOU + UOS = 3.58100 \times 2 = 7.16200$$

$$OOO = 12.37957$$

$$OOU = OOU + UOO = 6.66153 \times 2 = 13.32307$$

$$UOU = 3.58432$$

$$SUS = 2.84481$$

$$SUO = SUO + OUS = 5.29182 \times 2 = 10.58364$$

$$SUU = SUU + UUS = 2.84745 \times 2 = 5.69489$$

$$OUO = 9.84448$$

$$OUU = OUU + UOU = 5.29672 \times 2 = 10.59344$$

$$UUU = 2.85008$$

APPENDIX 7

SAMPLE CALCULATION FOR ALIGNMENT STRUCTURES OF TRIGLYCERIDE
MOLECULES IN REFINED OIL

$$SSS = 0.28469$$

$$SSU = SSU + USS + SSO + OSS = 0.56992 + 1.05915 = 1.62907$$

$$SUS = SUS + SOS = 2.84481 + 3.57769 = 6.4225$$

$$USU = USU + OSO + OSU + USO = 0.27755 + 0.98514 + 1.06013 = 2.32282$$

$$SUU = SUU + UUS + SOO + OOS + SOU + UOS + SUO + OUS =$$

$$5.69489 + 13.31073 + 7.16200 + 10.58364 + 36.75127$$

$$UUU = UUU + OOO + OOU + UOO + UOU + OUO + OUU + UUO =$$

$$2.85008 + 13.32307 + 3.58432 + 10.59344 + 9.84448 + 12.37957 +$$

$$52.57496$$

APPENDIX 8

COMPUTED VALUES FOR ALIGNMENT STRUCTURES OF TRIGLYCERIDE
MOLECULES IN REFINED OIL

$$\begin{aligned} \text{SSS} &= 0.086 \times 0.0810 + 0.123 \times 0.1955 + 0.368 \times 0.2795 + 0.52 \times \\ &0.4440 = 0.36475 \end{aligned}$$

$$\begin{aligned} \text{SSU} &= 0.878 \times 0.0810 + 0.930 \times 0.1955 + 2.428 \times 0.2795 + 1.871 \times \\ &0.4440 = 1.76385 \end{aligned}$$

$$\begin{aligned} \text{SUS} &= 2.581 \times 0.0810 + 4.176 \times 0.1955 + 5.057 \times 0.2795 + 12.256 \times \\ &0.4440 = 7.88056 \end{aligned}$$

$$\begin{aligned} \text{USU} &= 2.248 \times 0.0810 + 1.785 \times 0.1955 + 3.997 \times 0.2795 + 1.681 \times \\ &0.4440 = 2.39458 \end{aligned}$$

$$\begin{aligned} \text{SUU} &= 26.449 \times 0.0810 + 31.808 \times 0.1955 + 33.304 \times 0.2795 + 44.0615 \times \\ &0.4440 = 37.23269 \end{aligned}$$

$$\begin{aligned} \text{UUU} &= 67.188 \times 0.0810 + 60.7514 \times 0.1955 + 54.837 \times 0.2795 + 39.601 \times \\ &0.4440 = 50.22891 \end{aligned}$$

VITA

The author was born May 22, 1942, in Seoul, Korea. He graduated from Taejon High School in February 1960 and entered Seoul National University that spring. In August 1964 he received a Bachelor of Science degree in Liberal Arts and Science in Botany. He entered the same university in the spring of 1965 and obtained a Bachelor of Science degree in Chemistry in February 1971. During this time, he also served in the Korean Army as an Officer for 3 years. After graduation from the university, he worked in the Biochemistry Department in Catholic Medical College as an assistant, and also from 1972 to 1976, he taught chemistry in a high school in Seoul, Korea. In January 1977 he enrolled in the Graduate School of The University of Tennessee, Knoxville, and has been working toward a Master of Science degree in Agriculture with a major in Food Technology and Science.