Determination of Gossypol as an Undesirable Substance in Cotton Seed Cake and Oil of Different Cotton Varieties in Sudan

By

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قال تعالى:

﴿ قُلْ إِنَّ صَلاتِي وَنُسُكِي وَمَحْيَايَ وَمَمَاتِي لِلَّهِ رَبِّ الْعَالَمِينَ ﴾

صدق الله العظيم

سورة الأنعام – الآية (162)

DEDICTATION

To my parents

To my brothers and sisters

To my supervisor

To my friends

To all Muslims

ACKNOWLEDGEMENT

My Praise go to the a lmighty "ALLA" the most gracious and the most merciful who granted me the mind, health, strength and patience to conduct this study successfully. I indebted to my major supervisor, Dr. Salah Mohamed Nour for his fatherly advise, useful guidance, encouragement, Cooperation supervision and for the moral and professional support with which he provided me via the study. I am also indebted to my Co-supervisor Dr. Atif Abdel Moneim Ahmed for his advise during this study.

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ABSTRACT

Gossypol, a yellow pigment polyphenolic toxic compound. It's produced by some glands distributed all over the plant organs such as (Flower, bolls, seed, and leaves). It's concentrated in the seed appearing as dark dots. Gossypol has been recognized as a having adverse effect to human and animals if we found it in high concentration. The aim of this study was to determine gossypol in three different cottonseeds grown in Sudan plus seed of Ex1"experimental line" and their oil (Barakat 90, Abdein, Hamid and Ex_1"experimental line"). Cottonseed of four different varieties was obtained from Agricultural Research Corporation (ARC) Wad Medani. Several analysis were conducted: hundred seed weight, hulls to kernel, moisture and volatile matter, the oil content, extraction of oil, thin layer chromatography, high performance liquid chromatography. Previously analysis were according to american oil chemist society (A.O.C.S) methods. The results showed that: the weight of 100 seed were (11.02), (10.70), (10.44), (9.26) gram for Abdein, Barakat 90, Hamid and Ex_1 respectively; the kernel to hull ratio (wt%) were (1.43, 1.81, 1.92, 1.43) for Abdein, Barakat 90, Hamid andEx_1 respectively, the moisture content were (5.25, 5.17, 5.10, 5.28)% for Abdein, Barakat 90, Hamid and Ex_1 respectively, the oil content were (19.77, 20.78, 21.12 ,19.39)% for Abdein, Barakat 90 Hamid andEx_1 respectively, the mobility parameter (R_f) in a thin layer chromatography were (0.5, 0.6, 0.7, 0.8, 0.9), (0.2, 0.6, 0.6, 0.7, 0.9), (0.2, 0.6, 0.7, 0.8, 0.9), (0.2, 0.6, 0.7, 0.8, 0.9), (0.5, 0.6, 0.6, 0.7, 0.9) for Barakat 90, Hamid, Ex_1 and Abdein, respectively, the result of gossypol concentration as mean (%) in seed cake using high performance liquid chromatograph were (1.20±0.20, 0.76±0.17, 0.54±0.02, 0.31±0.02) for (Barakat 90, Ex_1, Abdein, Hamid) respectively. The result of gossypol concentration as mean (%) in oil using high performance liquid chromatography to extract gossypol from seed oil were (0.87±0.020, 0.60±0.020, 0.40±0.020, 0.27±0.015)% for (Barakat 90,Ex_1, Abdein, Hamid) respectively. This study recommend that Hamid and Abdein variety is much recommended to be used for oil production for its low gossypol content as Barakat 90 should be treated and refined or extracted with optimum method to raise the oil content and to reduce gossypol content.

تحديد الجوسيبول كمادة غير مرغوب فيها في أربعة عينات بذور قطن وزيت البذور بالسودان

آيات عبد الحميد أحمد عبد الحميد
ماجستير الأغذية في علوم الأغذية (فبراير، 2013)
قسم علوم وتكنولوجيا الأغذية
كلية الهندسة والتكنولوجيا
جامعة الجزيرة

الخلاصة

الجوسيبول مادة صفراء عديدة الفينولات يتم إنتاجها بواسطة بعض الغدد التي تتوزع على كل أجزاء نبات القطن مثل (الأوراق، البذور، البراعم، الأزهار) وتتركز في البذور وتظهر في شكل نقاط سوداء، وقد عرف بأن له تأثيرات سالبة على الإنسان والحيوان إذا وجد بتركيزات عالية. هدف هذا البحث إلى تحديد كمية الجوسيبول الموجودة على بذور وزيوت ثلاثة أصناف مختلفة من القطن المزروع في السودان بالإضافة إلى Ex_1 "صنف جديد" (بركات 90، عابدين، حامد وEx_1 "صنف جديد") في السودان. تم جمع بذور القطن للأصناف الأربعة من هيئة البحوث الزراعية بود مدني حيث تم إجراء التحاليل التالية: وزن ال 100حبه، نسبة القشرة إلى النواة، الرطوبة والمواد الطيارة، محتوى الزيت، تحديد الجوسيبول بواسطة كروماتوغرافيا الطبقة الرقيقة، تحديد نسبة الجوسيبول في البذور والزيت باستخدام كروماتوغرافيا الضغط العالي . الطرق الكيميائية المتبعة في التحاليل آنفة الذكر هي الطرق الموصى بها من قبل الجمعية الأمريكية لكيميائيي الزيوت، وكانت النتائج كالآتي: كان وزن ال 100حبه بالجرام (11.01، 10.70، 10.44، 9.26)% وكان نسبة النواة إلى القشرة (1.43، 1.92، 1.81، 1.43)% للأصناف عابدين، بركات 90، حامد وEx_1 على التوالي، ومحتوي الرطوبة كان (5.25، 5.17، 5.10، 5.28)% عابدين، بركات 90، حامد وEx_1 علي التوالي، وكان محتوى الزيت (19.77، 20.78، 21.12، 19.39) % للأصناف عابدين، بركات 90، حامد وEx_1 على التوالي، معامل الإعاقة لكروماتوغرافيا الطبقة الرقيقة كان (0.5، 0.6، 0.7، 0.8، 0.9)، (0.2، 0.6، 0.6، 0.7، 0.9)، (0.2، 0.6، 0.7، 0.8، 0.9)، (0.2، 0.6، 0.7، 0.8، 0.9)، (0.5، 0.6، 0.6، 0.7، 0.9) للأصناف عابدين، بركات 90، حامد وEx_1 على التوالي، متوسط نسبة الجوسيبول المستخلص بواسطة كروماتوغرافيا الضغط العالي كان(1.20±0.02، 0.76±0.12، 0.54±0.17، 0.31±0.02)% للأصناف بركات 90، Ex_1، عابدين وحامد على التوالي، وكان متوسط نسبة الجوسيبول المستخلص من الزيوت بواسطة كروماتوغرافيا الضغط العالي (0.87±0.02، 0.60±0.020، 0.40±0.020، 0.27±0.015)% للأصناف بركات 90، Ex_1، عابدين وحامد على التوالي. توصي الدراسة باستخدام الأصناف حامد وعابدين لإنتاج الزيت لاحتوائها على نسبة أقل من الجوسيبول مقارنه ببركات90 الذي يجب معالجته وتكريره بالطرق المثلى لزيادة محتوى الزيت وتقليل نسبة الجوسيبول.

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LIST OF ABBREVIATIONS

ABBREVIATION	DIFINITION
ANOVA	Analysis of Variance
ARC	Agricultural Research Corporation
BG	Bound Gossypol
CPFA	Cyclopropen Fatty Acid
DF	Degree of Freedom
CS	Cotton Seed
DNA	Deoxy ribo Nucleic Acid
EFC	Extra Fine Stable Cotton
FDA	Food and Drug Administration
FG	Free Gossypol
FSH	Follid Stimulated Hormone
GJIC	Gap Junction Intercellular Communication
GST	Glutathione S-Transferase
HA	High Acala
HAP	Hazardous Air Pollution
HPLC	High Performance liquid Chromatography
HSD	Hydroxyl Steroid Dehydrogenase
IV	Iodine Value
KOH	Potassium hydroxide
LD50	Lethal Dose
NAOH	Sodium hydroxide
NOPRI	National OilSeed Processing Research Institute
Pc	Prostate cancer
PBSY	Prime-Bleachable Summer Yellow
R_f	Mobility parameter

SC	Short count
SCC	Sudan Cotton Company
Sig	Significant
ST.E	Stander Error
ST. Dev	Stander Deviation
TBHQ	Tertiary-Butyl Hydro Quinone
TGF	Transforming Growth Facter
TLC	Thin Layer Chromatography
USA	United State of America
UV	Ultra-Violet
V/V	Volume per Volume
VOC	Volatile Organic Compound
WCS	Whole Cottonseed

CHAPTER ONE

INTRODUCTION

1.1 Background

Cotton is an important commercial crop of Sudan. It's represent the main cash crop which insure the foreign currency required to purchase most of the agricultural inputs.In Sudan, the contribution of cotton alone to the National economy ranged between 45% and 65% of the total foreign currency earnings (Sudan Cotton Company: SCC, 1993). In addition, cotton is considered as a main source of income for 13% of total labour force (Adam and Kuhlman, 2002). Total area under cotton in Sudan in 1972 was 1.209.634 feddan,from 1972-2002 the area under cotton, yield and production dropped on average, by 38, 18 and 48% (Ahmed *et al.*, 2004). The infestation by major cotton pests such as the African bollworm, and others, limits cotton production in Sudan (Mathews, 1987).

There are many obstacles facing the full utilization of the cotton crop, especially if we consider the cottonseed oil as a target where it is used to produce edible oil as well as seed cake for animal feed. One of the most important problems here is that the seed contains some undesirable chemicals, specifically gossypol and it's derivatives which represent the main component of the plant pigments.Gossypol is toxic compound produced by some pigment glandsPigment glands are distributed in cotton plant parts above ground (Lukefahr *et al.*, 1966) It is found in two type of glands, i.e internal and external which are usually known as nectar or pigment glands (Moore and Rollin, 1974) These glands are found in the leaves, stems, roots, and seeds of cotton plants (Boatnerand Charlotte, 1948) and the epidermal glands of cotton plant, (Quainintance and Brues,

1905; Cook, 1906). The absence of pigment glands does not ensure elimination of gossypol, (Fisher *et al*., 1988). High concentrations of these substances are harmful to human and animal, especially when they exceed the Food and Drug administration of the USAFDA safe level 0.45% as free gossypol (FG) (Cherry, 1983).This gossypol significantly drew the attention because of it is toxicity and also for being localized in the seeds having nutritional value, i.e high oil and protein contents (Moore and Rollins, 1974).

1.2 Objectives:

The objectives of the present study include:

1- To determine gossypol in cottonseed.

2- To determine gossypol in cotton seed oil.

CHAPTER TWO

LTERATURE REVIEW

2.1 Cotton:

Cotton belongs to genus,*Gossypium,* family *Malvaceae,* which contains 50 different species (Mohamed, 2003). The tribe *Gossypieae* and the genus*Gossypium* that grows naturally as a perennial but for commercial purposes is grown as an annual (Wakelyn, 2002).There are four Gossypium species namely *G. arboeum, G. herbaceum, G.barbadense* and *G. hirsutum* are cultivated throughout the world (Innes, 1983).

Cotton had been cultivated in tropical and subtropical climates of the world since prehistoric time (Munro, 1994). The countries such as USA, India, China, Egypt, Mali, Sudan and Zimbabwe (El Bashir, 2000).The total area under cotton cultivation in the world was about 31 million hectares producing around 20 millions metric tons (I.C.A.C, 2001).Planting time for cotton varies by locality, varying from February to June in the Northern Hemisphere. Harvest time is in the late summer or early late fall.In the western Hemisphere, cotton is cultivated between about 37° N and 32°S latitude and in the eastern hemisphere, between about 47°N and 30°S (Kohel and Lewis, 1984).

2.1.1 Cotton in Sudan:

Cotton is the backbone of Sudan economy and it is contributes by more than 40% to Sudan export values, it ranks first among the main cash crops (Elnour, 2000).

Commercial Sudanese cotton varieties include the extra long staple cotton (extra fine count cotton (EFC), Barakat, Barakat 90, and Barakat S, the high medium staple cotton (High Acala Count Cotton HA), Nour, the medium staple cotton (Short Count (SC), Acrain and Albar (57) 12 (Nuba Albar), (SCC. Technical section, 2005).Sudan is the world second largest producer of extra long staple cotton after Egypt (Fadlalla, 1990; Elamin, 1997).

Sudanese cotton production began in 1862 with the introduction of cotton plant into the Tokar area of Eastern Sudan. However commercial production began in 1905 in Zeidab pilot scheme in Northern Sudan. In 1911, cotton was planted on an experimental scale at Tayiba (North Madene) Gezira State, as a precursor to Gezira scheme. After that, cotton planting was widely spread in irrigated scheme (Gezira, New Hlafa, Rahad, and Suki) and in permanent irrigation by pump (Blue Nile, White Nile) and by flood and rainfall (Northern and Sourthern Kordefan, Damazeen and Gadareef) and by spate irrigation (Tokar and Gash). (Tijani and Elbashir, 1977; GSB Publication, 1971; AdelBagi, 2003).

2.2 Cottonseed oil:

Cottonseed oil is a cooking oil extracted from the seeds of cotton plant of various species, mainly *Gossypium hirsutum* and *gossypium* herbaceum, Cottonseed is about 15 – 20% of the value of the cotton crop (Anonymous, 2000) it contain 2% oil and 23% protein of relatively high quality (Stipanovic, 1999).

A typical cottonseed crushing operation will separate the seed into oil (160 kg/t, 320 lbs/t), hulls (260 kg/t (450 lbs/t), meal (455 kg/t (910 lbs/t)), and linters (835 kg/t (167 lbs/t)) (Anonymous, 2000).The hulls and meal are sources of vegetable protein feed for animals and the linters are used as

chemical cellulose source in personal care products, in battling for upholstered furniture and mattresses, and in high quality paper (Gregory *et al*., 1999).

Cottonseed oil, Americas original vegetable oil, dominated the united states vegetable oil market for almost 100 years, the English and European vegetable oil industry was based on variety of oilseeds and tree fruits available in the home countries and their colonies. Cottonseed oils properties have helped to maintain it as an important source oil for food product world wide and made it the standard to which other oils are compared (Wakelyn, 2002). The scientific and technical advance developed to process cottonseed became the known to day.

2.3 Cottonseed handling; oil extraction and processing:

Nature has provided plants with systems to synthesize, utilize and store food lipids. Improper handling and storage of oilseed prior to extraction can have deteriorous effects on the oil quality. Therefore, control of cottonseed transportation, storage, segregation of lots, and moisture are the first processes for processing a desirable edible oil (Johnson,1981).

Oil extraction has a long development history, while most of the oil processing methods were largely introduced during the twentieth century. Until the recent past, crude oil extraction and oil processing were two separate industries. However, during the last quarter century, sheer economics and product synergy have caused both horizontal (merger of similar operations) and vertical integration (combination of different but related operations) of these activities to occur. Now companies continue to increase their crushing capacity and many extract and refine the oil as a continuous operation. Most of these operations have integrated miscella refining with sodium hydroxide to produce a prime-bleachable-summer-

yellow (PBSY) cottonseed oil with consistently lighter color. The caustic-refined cottonseed oil, is a trading definition of the National Cottonseed Products Association with an AOCS official bleach color of no greater than 2.5, no more than 0.25% free fatty acid, and no more than 0.10% moisture and volatile matter. The oil processor is guaranteed quality via this trading rule with its 2.5 laboratory bleach color (Johnson,1981).

2.3.1 Cottonseed handling and storage:

Once a cotton boll opens, the cottonseeds within are susceptible to deterioration. The living seeds respire, producing carbon dioxide and heat. Other biological processes occur in the seeds as well. Triglycerides are split by enzymes via hydrolysis to release free fatty acids (FFA), which the embryo plant uses for energy. The production of free fatty acids is undesirable for the oil processor because seeds high in FFA contain low-quality oil, which usually results in a higher refining loss. The rate of hydrolysis is dependent on temperature and moisture, but the hydrolytic enzymes may be inactivated by heat. Oxidation of the fatty acids in the oil can result from heating (Johnson,1981). (Altschul,1948) comprehensively reviewed the biological processes that take place in the cottonseed before and after harvest, and more recent reviewers (Mauney and Stewart, 1986) have considered cottonseed development.Another factor in cottonseed storage is the control of mold development on the seeds. Cottonseed is particularly susceptible to the fungus *Aspergillus flavus* (Park *et al*., 1985). Ideally, cottonseed should be stored at a moisture content of less than 10% (Norris, 1982). Dehulled seeds should contain no more than 9% moisture and 1% FFA (Johnson,1981). Prior to storage, cottonseeds must be sampled for moisture analysis. Seeds with 10–11% moisture may be stored immediately, but seeds with higher moisture require additional drying.

Drying may take place at either ambient temperatures or up to 104.5°C (220°F), to 12% or less. There is no benefit to drying the seeds below 9% moisture. Although dryers may not be needed every season, they are a necessity when wet seeds are delivered to the mill (Norris *et al*., 1958).As the seeds are hygroscopic, the relative humidity of the air in storage must be monitored to maintain the desired seed moisture level. (Rhee *et al*., 1988).

The most common type of cottonseed storage facility is the seed house, an air-cooling system is vital to the successful storage of cottonseed. The temperature of the seeds is dependent on the ambient temperature and degree of ventilation in the storage area (Rusca and Gerdes, 1948). As the seeds are respiring, heat can build up, particularly if the seeds have a high moisture or FFA level (Norris, 1982). Over heated seeds must be cooled to below 60°F (15.6°C) to prevent further deterioration (Alderks, 1948).

2.3.2 Cottonseed oil extraction:

Cottonseed was one of the earliest oils to be extracted from the seed. The extraction of cottonseed oil slowly progressed from edgestone to wedge press to hydraulic press (Bailey, 1948).Hydraulic pressing was the predominant means used to separate the oil from cottonseed for most of the nineteenth century. As improved cotton spinning, weaving, and ginning operations during the eighteenth century made more cottonseed available for crushing, the labor-intensive hydraulic-press operation quickly yielded to the continuous screw press in the early 1900s. As edible oil commanded a respectable market price and because either the hydraulic press or the screw press still left nearly 20% of the available oil of the seed in the press-cake, much research activity was initiated to find an acceptable solvent to extract the remaining oil from the cake. This effort led to the prepress

solvent extraction process in the 1930s. This combination of mechanical press and solvent extraction of the press-cake was able to recover better than 97% of the available oil in the cottonseed (Brien and Wan, 2001).

The flow sequence for cottonseed oil extraction illustrated in Figure (1) (Wakely, 2001).

2.3.3 Cottonseed preparation:

Most oilseeds require some degree of cleaning and preparation before the oil is separated from the solid portion of the seed. After the cotton fiber is removed from the seed by the ginning operation, the seed still has short linters with a white appearance. This is called white or fuzzy cottonseed in the trade. On a dry basis, white cottonseed is composed of 12.7% linter, 31.8% hull, and 55.5% kernel (Bailey, 1948).

As illustrated in Figure (1) cottonseeds are usually stored uncleaned when received at the oil mill. When the cottonseed are removed from storage for extraction, dirt and other trash must be removed. Several seed cleaning systems are used, which are all based on some type of screening. Trash that is lighter or smaller than the seeds will be aspirated in pneumatic systems or sifted out mechanically. The larger pieces of trash are screened out and magnets are used to remove ferrous metal. Foreign materials that have the same size and density as the seed can still be carried on through the process stream (Gallowayand Amer, 1976).

2.3.3.1 Delinting:

This step is unique to cottonseed among all the oilseeds. The short cellulose linter fibers must be removed from the seed because leaving them on the seed would lower the yield of oil due to absorption of the oil by the cellulose fibers. Linters, are bulky and tend to hold the neutral oil or

occupy valuable extractor space during the extraction. Chemicals, such as sulfuric acid, have been used to remove the linters, especially when seeds are prepared for replanting. However, all commercial mills remove the linters mechanically as these short fibers have many nonfood applications, serving as the starting material for pure cellulose, plastics, and rayon and are used in high-quality paper, batting or padding in bedding and furniture, and automotive uses (Alderks, 1948).

2.3.3.2 Hulling:

Once the lint is removed, the hulls are separated from the seeds. Hulls that are allowed to remain with the kernels absorb oil during extraction and lower the quality of the meal produced by lowering the protein level. The hulls can not be completely eliminated without a loss of kernel, so an acceptable level of hull retention must be determined, depending on the desired protein level of the final meal(Alderks, 1948).

Two types of hullers are used in the industry. The bar huller consists of a bar- or knife-studded cylinder that rotates within another cylinder having similar knives protruding from its interior. The hulls are cut as the seeds pass around the inner cylinder. The seed decorticator has two hardened steel rolls, both of which have longitudinal grooves cut in the surface. The seeds are fed between the rolls and then cut by the grooves and the difference in speed between the two rolls. The hulls and uncut seeds are removed from the kernels by screening and the hulls are aspirated so that the seeds may be returned to the huller (Alderks, 1948).

2.3.3.3 Flaking:

After hulling,the meals, or kernel, are reduced in size or flaked to facilitate oil removal. This rolling process minimizes the distance through

which the free oil must pass, but it does not necessarily rupture the walls of the oil cells. Proper moisture content of the seeds is essential for flaking, and if the moisture level is too low, the seeds must be conditioned to raise the moisture to about 11% (Alderks, 1948).

Cottonseed may be flaked by passing between two rolls mounted side-by-side; however, they are more often flaked in a series of five stacked-crushing rolls because a thinner flake may be achieved with the vertical rolls. The ultimate thickness of the flake is determined by the method of extraction used.

For mechanical pressing, a thickness of 0.127–0.254 mm (0.005–0.010 inch) is common, and for solvent extraction, flakes of not less than 0.230–0.254 mm (0.008–0.010inch) are common. Thinner flakes tend to disintegrate during the solvent process(Norris, 1982).

2.3.3.4 Cooking:

Prior to extraction, the flakes are heated or cooked because:

a) cell walls are broken down allowing the oil to escape.
b) Oil viscosity is reduced.
c) Moisture content is controlled.
d) Protein is coagulated.
e) Enzymes are inactivated and micro-organisms are killed;. Gossypol is bound to protein, to some extent, by the action of heat in combination with moisture and physical treatment and thus some portion of it is detoxified.
f) Certain phosphatides are fixed in the cake, which helps to maintain subsequent refining losses(Norris, 1982).

Cottonseed flakes are usually cooked in stack cookers that are 4–8 kettles high. The sides and floors of each kettle are steam-jacketed to heat

the flakes. The flakes are fed into the top kettle, heated for a specific time, and then swept into the kettle below. The temperature of lower kettles are usually maintained at higher temperatures than the top kettle. If the flakes are relatively dry, moisture may be added to the top kettle to reach a level of 11–12%. As the flakes progress toward the bottom kettle, water is evaporated and removed by vents in each of the lower kettles until the final moisture level is reached. The desirable level is 5–6% moisture for seeds to be hydraulically pressed and about 3% for seeds to be expeller or screw pressed (Norris, 1982).

The flakes are heated to over 190°F (87.8°C) in the upper kettle. Flakes with high phosphatide content may benefit from being cooked at slightly lower temperatures to avoid elevating refining losses, The temperature of the flakes is raised to 230–270°F (110–132.2°C) in the lower kettles. The seeds are cooked for up to 120 minutes and, depending on the size of the cooker, 81–136 metric tons (90– 150 short tons) of meats may be cooked in a 24-hour period(Brien and Wan, 2001).

Overcooking lowers the nutritional quality of the meal and darkens both the oil and the meal. Poor-quality seeds with high levels of free fatty acids cannot be cooked for as long a period as high-quality seeds because of darkening. Darker oil requires additional refining to achieve the desired bleach color (Brien and Wan, 2001).

2.3.4 Oil extraction:

Four types of processing systems are used to extract oil from oil-bearing materials:

a) Hydraulic press.
b) Expeller or screw press.
c) Prepress solvent extraction.

d) Direct solvent extraction.

These systems employ the two techniques in common practice for the extraction of cottonseed oil. These are mechanical by means of a press or the solvent process with the use of hexane. Mechanical pressing is normally applied to oilseeds that are relatively high in extractable oil. Hull-free cottonseed kernels contain as much as 34% oil and are suitable for the mechanical extraction process (Brien and Wan, 2001). The prepress solvent system employs a combination of the two techniques, where seeds are lightly screw pressed to reduce the oil by one-half to two-thirds of its original level before solvent extraction completes the job.On a world wide basis, due to available transportation infrastructure, hardware, solvent, and skilled labor, cottonseed is still being processed with all four extraction systems (Wakely, 2001).

2.3.4.1 Hydraulic pressing:

Batch pressing was the earliest commercial method of oil extraction. Hydraulic equipment replaced the mechanical operations and the method became known as hydraulic pressing, in this presses, oilseed meals were wrapped in cloths and placed between plates, which were then gradually compressed to squeeze the oil from the seeds. Box-type presses were most often used for cottonseed, and this method was fairly labor intensive. Today, very little cottonseed is hydraulically pressed(Wakely, 2001).

2.3.4.2 Screw pressing:

The screw-press are commonly used for pressing cottonseed oil. pressure is gradually applied to the flakes as a screw conveys them from the feed end to the discharge end of the expeller barrel. A plug of the compressed meal develops at the discharge end and a drainage barrel

surrounds the press to collect the oil expressed during the passage of the flakes. About 3–4% oil remains in the cake that results from screw pressing (Norris, 1982).

Anderson (Cleveland, Ohio) expellers have both vertical and horizontal presses to maximize pressure, and French (Piqua, Ohio) screw-presses generally consist of a horizontal, water-cooled cage. Both types of presses exert 680–1089 atm (5–8 tons per square inch) pressure on the flakes (Wakely, 2001). A disadvantage of the screw press method compared with the now outdated hydraulic pressing was the tendency of screw pressed cottonseed oil to have higher color due to the lower moisture content of the cooked meats prior to pressing (Norris, 1982).

2.3.4.3 Direct solvent:

This process is based on the use of a nonpolar solvent, specifically hexane, to dissolve the oil without removing proteins and other compounds. The flakes are mixed with hexane in a batch or continuous operation. The resulting oil-solvent micelle and the residual meal are heated to evaporate the solvent, which is collected and reused (Wakely, 2001)..

Solvent extraction yields about 11.5% more oil than does the screw-press method (Wakely, 2001). and 1% or less oil remains in the meal. However, direct solvent extraction is problematic for the cottonseed industry because the high oil content of cottonseed flakes causes them to break into fires during extraction after the oil is removed; occasional overheating of the oil-solvent miscella will cause irreversible color changes in the oil; the solvent poses fire problems and is expensive; hexane poses environmental pressures as a volatile organic compound (VOC); and the main component of hexane, n-hexane, is also classified as a Hazardous Air

Pollutant (HAP) by the U.S. Environmental Protection Agency and is strictly regulated in the United States (Wakely, 2003; Wan and Wakelyn, 1998).

2.3.4.4 Prepress solvent extraction:

Solvent extraction is relatively costly and is not well suited for the high-oil content of cottonseed. Mechanical pressing leaves about 5% oil in the press-cake, and it is desirable to recover as much oil as possible. A logical processing step was to combine the two extraction techniques. With prepress solvent extraction, cottonseed are pressed to remove most of the oil and then the oil remaining in the press-cake is extracted with solvent. This solvent extraction operates on a reduced volume of feed stock (i.e., press-cake, as opposed to full-fat flakes) and, therefore, requires a modest size extractor with modest amounts of desolventizer and solvent (Carlson, 1991).

2.3.4.5 Expander-solvent extraction:

The most recent development in cottonseed extraction is the use of expanders. The expander is a low-shear extruder that heats, homogenizes, and shapes oilseeds into porous collets or pellets with a high-bulk density. Steam is injected into the oilseed flakes or cake in the expander while under pressure, and then this mixture is extruded through plates to the atmosphere, the collets expand when released to the atmosphere, hence the name expander. (Carlson, 1991).

Some expanders have a drainage cage to reduce the oil content of high-oilseeds to less than 30%, thus enabling the production of intact collets for direct solvent extraction, instead of the prepress extraction process (Carlson, 1991). Watkins (Watkins, 1988) reported that after hexane

extraction, the collets had 25–45% less solvent holdup and 15 times less oil than did traditionally prepared flakes. Solvent capacity is enhanced because the extruded cottonseed requires solvent-feed ratios of 1:1, compared with 1.8:1 for direct extraction of cottonseed meats (French, 1988).

After extraction, the meal contains 0.10–0.20% free gossypol, which is about half the amount found in flakes. As of 2000, essentially all commercial cottonseed extraction in the United States employs the expander solvent extraction process (Brien, 1998).

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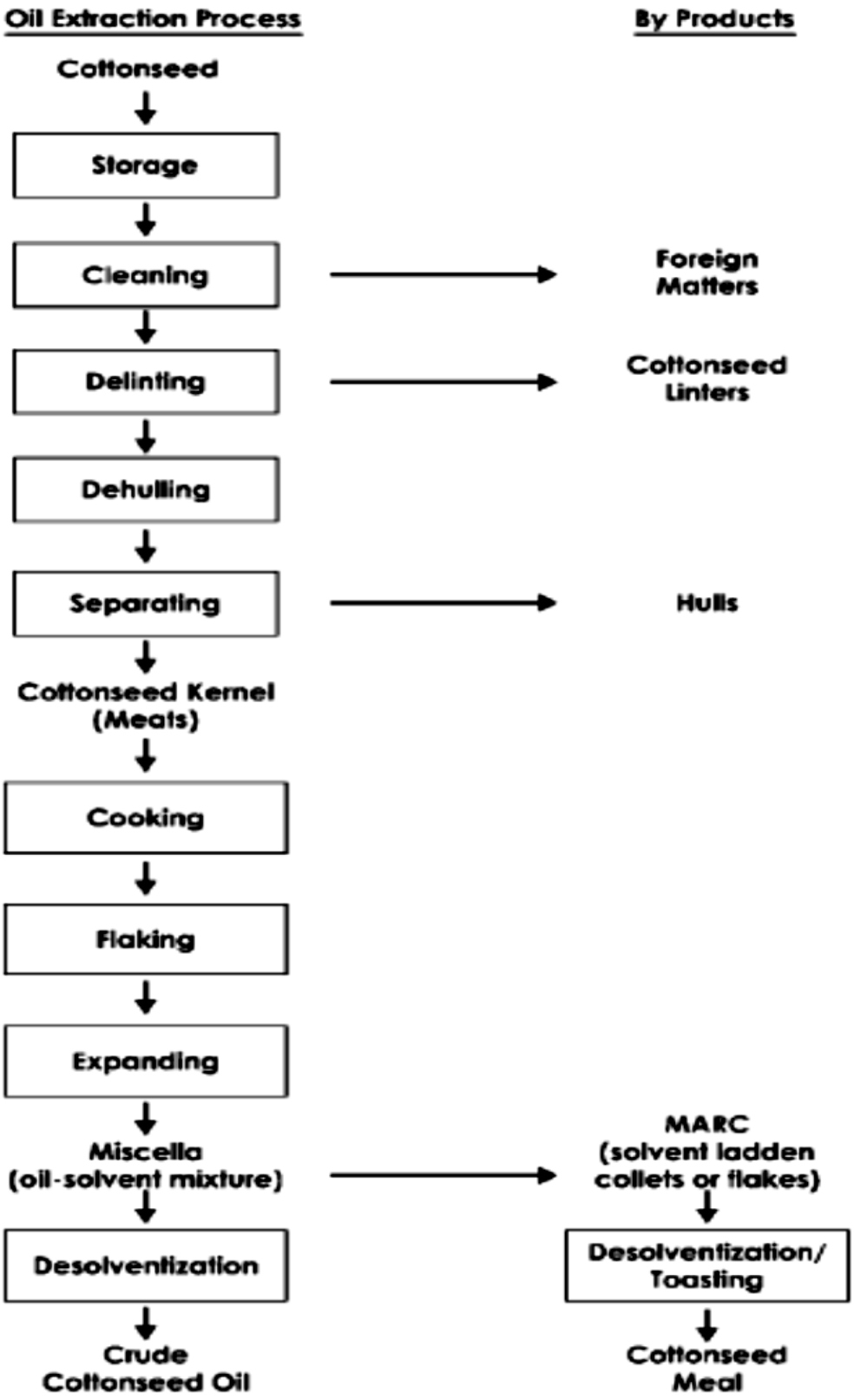

Figure(1): Cottonseed oil extraction flow sequence

2.3.5 Cottonseed oil processing:

Processing flow sequences for six different product groups are illustrated in Figure (2). Cottonseed oils need many processes after extraction because consumers have been conditioned to prefer edible oils that are light in color, bland flavored, have a high smoke point, maintain a clear appearance both on the grocery shelf and under refrigeration, contain additives to prolong flavor and frying stability, are modified to provide a specific performance characteristic, and attractively packaged for convenient handling.The processes responsible for these and other oil product qualities are called refining(Brien, 1998).

2.3.5.1 Refining:

The term refining refers to any purification treatment designed to remove FFA, phosphatides, and any gross impurities in cottonseed oil, it excludes any other process, such as bleaching or deodorization. The refining process probably has more impact on a vegetable oil's quality and economic performance than any of the other processes during the conversion to a finished product. Inadequately refined oils will affect the operation of all succeeding processes and the quality of the finished product. Additional processing and handling required because of poorly refined oils will increase the costs of a suspect quality finished product beyond that of one produced with a properly refined oil that has a good quality(Brien, 1998):

Two different refining systems are currently used to refine vegetable oils; chemical and physical refining. Some oils, like cottonseed, contain nonglyceride materials that cannot be removed adequately by the processes employed with physical refining. nonglyceride materials in cottonseed oil readily combine with caustic soda and, thus, are removed most effectively

by alkali-refining, several different versions of the alkali-refining process are practiced in the United States and other countries: long-mix, short-mix, miscella, and the Zenith process (Brien, 1998).

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2.3.5.2.1 Conventional or Long-mix caustic-soda refining:

The conventional caustic soda refining process is the most widely used and best known refining system. The addition of an alkali solution to a crude oil brings about a number of chemical and physical reactions. The alkali combines with the free fatty acid present to form soaps. The phosphatides and gums absorb alkali and are coagulated through hydration or degradation. Much of the coloring material is degraded, absorbed by the gums, or made water soluble by the alkali, and the insoluble matter is entrained with the other coagulable material. With heat and time, the excess caustic can also bring about the saponification of the neutral oil. Therefore, selection of the NaOH strength, mixing time, mixing energy, temperature, and the quantity of excess caustic all have an important part in making the alkali-refining process operate effectively and efficiently (Brien,1998).

The current alkali-refining techniques are a result of the gradual application of science to the basic art of batch refining originally performed in open-top, cone shaped kettles. Efficient separation of soap stock from the neutralized oil is the significant factor in alkali refining, and the technique of using centrifugal separators materially improved the yield from 1.5% to 2.5% (Brien,1998).

2.3.5.2. Pre pleaching:

Bleaching is popularly and correctly regarded as the partial or complete removal of color; however, it is also a purification process to prepare the oil for further processing. Bleaching is relied on to clean up the traces of

soap, phosphatides, and pro-oxidant metals remaining after caustic neutralization and water washing that hinder filtration, poison hydrogenation catalysts, darken the oil, and adversely affect the flavor of the finished oil. Another function, considered primary by many processors, is the removal of peroxides and secondary oxidation products (Brien,1998). The key process parameters for bleaching include:

a) Procedure.
b) Bleaching media.
c) Temperature.
d) Time.
e) Moisture.
f) Filtration (Brien,1998).

2.3.5.2.1 Procedure:

The three most common types of contact bleaching methods are batch atmospheric, batch vacuum, and continuous vacuum. Although vacuum bleaching is preferred, atmospheric bleaching can produce quality bleached oils. Vacuum bleaching, either batch or continuous, is more effective than atmospheric bleaching because it can use less clay, operates at lower bleaching temperatures, effects quicker moisture evacuation for less FFA development, and does not expose the oil to oxidation at high temperatures. Batch bleaching is preferred to continuous operations when a variety of source oils are processed in the same system. However, continuous systems are more efficient and effective for systems dedicated to single source oils (Norris, 1982).

2.3.5.2.2 Bleaching agents:

Chemical agents have been proposed and some used, but practically all edible oil decoloration and purification is accomplished with adsorptive earths or carbons. The basic kinds of adsorbents used in edible oil bleaching are neutral clays, activated earths, and activated carbon. Particle size is a major physical parameter affecting bleaching earth performance because adsorption theory considers adsorption as a surface phenomena. In general, the finest particle size earths have the best performance; however, too small particles create severe filtration problems and oil retention is increased.(Norris, 1982).

The natural bleaching earths, usually referred to as ‘‘fullers earth’’ are bentonite clays that exhibit adsorptive properties in the natural state with only physical processing. Activated bleaching earths have been treated with organic or inorganic acids to enlarge the surface and pore volume to make it selectively attractive to the detrimental components in refined oils. Also, the activated bleaching earths normally contain 10% to 18% moisture, which supports the montmorillonite layers in the clays. Activated carbon is effective in adsorbing certain impurities not affected by earths, but it is used sparingly due to problems with filtration, relatively high cost, and a high oil retention (Norris, 1982).

2.3.5.2.3 Bleaching media dosage:

The amount of bleaching material used depends on the type of adsorbent used and the impurities to be removed. Bleaching earth requirements vary in wide range from 0.15% to 3.0%. On the basis of adsorbent activity, the acid-activated earths are generally 1.5 to 2 times more effective than the natural earths. Carbon is rarely used alone but

sometimes employed in admixture with a bleaching earth in a ratio of 10–20 parts bleaching earth to 1 part carbon (Norris, 1982).

2.3.5.2.4 Temperature:

Bleaching earth activity increases as the temperature is increased by reducing the viscosity of the oil, but decoloration declines after the optimum temperature is reached. Temperature also affects other properties of the oil, which dictate that it should be kept as low as possible to minimize product abuse, but high enough for adequate absorbance of the impurities and pigments. The optimum bleaching temperature for nearly all edible fats and oils ranges between 70°C and 110°C or 160°F to 230°F (Weiss, 1983).

2.3.5.2.5 Time:

In theory, adsorption should be practically instantaneous however, in practice, this is not the case. The rate of discoloration is very rapid during the first few minutes after the adsorbent comes in contact with the oil and then decreases to a point where equilibrium is reached and no more color is adsorbed. Usually 15 to 20 minutes contact time is adequate at a bleaching temperature above the boiling point of water. Contact time is made up of two time periods:

a) The time in the bleaching vessel .
b) The time in the filter during recirculation or final filtering.

2.3.5.2.6 Filtration:

Filtration, the separation method most often used for spent bleaching media removal, is the process of passing a fluid through a permeable filter material to separate particles from the fluid (Weiss, 1983).

2.3.5.3 Winterization:

When cottonseed oil is designed for use as salad oil, it must be winterized, that is, a considerable portion of the more saturated glycerides must be removed so that the material will remain clear when exposed to reduced temperatures, such as those likely to be encountered with refrigeration. If the saturated glycerides in cottonseed oil are not removed, it will solidify at temperatures encountered in a refrigerator; 45°F or 7.2°C (Weiss, 1983).

Winterization is a narrow form of fractionation. Both fractionation and winterization processing operations for edible oils basically consist of the separation of oils into two or more fractions with different melting points. In the winterization process, the oils are cooled in a simple way, kept at the low temperatures for some time to crystallize solid-fat fractions that would normally cloud when the oil is held at refrigerator temperatures, and generally separated by filtration. With fractionation processes, cooling of the oil and the separation of the fractions are performed with more sophisticated techniques and controlled conditions to provide substances with unique properties (Bland, 1991).

The descriptive term of winterization evolved from the observation that refined and bleached cottonseed oil stored in outside tanks during the winter months physically separated into clear and hard fractions. Topping or decanting the clear oil from the top of the tanks provided an oil that remained liquid without clouding for long periods at cool temperatures. A need for a liquid oil with these characteristics was created by the introduction of the refrigerator for home use and the requirements of the mayonnaise and salad dressing industry. The indoors process developed to simulate the natural winter process consisted of a chilled room held at 42°F or 5.6°C, with deep, narrow, rectangular tanks to provide the maximum

surface exposure to cooling. Warm, dry, refined, and bleached cottonseed oil pumped into the chill room tanks began to cool and crystallize out stearine immediately but slowly. Convection heat transfer simulated the outside storage conditions (Bland, 1991).

Agitation was avoided because it fractured the crystal, causing formation of small, soft crystals that were difficult to filter. Cooling with the 42°F or 5.6°C, at room temperature, which simulated mild winter conditions closely, required 3 days to produce the desired large crystals for filtering. After the oil temperature equated with the room temperature, it was held for several hours to allow the stearine or hard fraction to precipitate more fully. The stearine was separated from the liquid oil by filtering with plate and frame presses. Normally, the oil was gravity fed to the filters to avoid breaking up the crystals. Winterization is still performed with the classic technique described above. However, most processors have made equipment and process modifications to improve efficiency; such as jacketed, enclosed tanks equipped with programmable cooling and agitation, better filtration, improved pumping methods, and so forth (Bland, 1991).

2.3.5.4 Fractionation:

In the fractionation process, the minor components of the original oil become concentrated in the separated fractions. This concentration has a considerable effect on the oxidative stability of the individual fractions. Relative to the starting oil, the liquid or soft fraction is enriched in tocopherols and depleted of trace metals. The reverse occurs with the hard or stearine fraction , which becomes appreciably more susceptible to oxidation despite its lower content of unsaturated. The stearine fraction is

also the recipient of other impurities remaining in the oil after refining and bleaching, such as phosphatides and soap (Cavanagh, 1961).

There are three processes in commercial use for the fractionation of edible fats and oils:

2.3.5.4.1 Dry fractionation:

The principal of this fractionation process is based on the cooling of oil under controlled conditions without the addition of chemicals or solvents. The liquid and solid phases are separated by filtration (Cavanagh, 1961).

2.3.5.4.2 Detergent Fractionation:

The oil is crystallized on its own similar to the dry fraction technique, but separation is affected by employing an aqueous detergent solution and centrifugation (Cavanagh, 1961).

2.3.5.4.3 Solvent fractionation:

The separation of component triglycerides that differ in solubility is accomplished by fractional crystallization of a solution of oil in an organic solvent, followed by separation of the solids from the liquid by filtration, and finally, removal of the solvent from the separated fractions by steam stripping. This process is the most versatile of the fractionation techniques presented (Cavanagh, 1961)..

Fractionation systems a considerably lower viscosity, which allows a faster crystal growth for more rapid stearine separation and the cottonseed salad oil produced also has a better resistance to clouding at cool temperatures for longer cold tests; and less liquid oil is trapped in the stearin component for higher salad oil yields (Cavanagh, 1961).

Fractionation technology, in particular solvent fractionation, has been utilized to produce some very highly specialized edible oil products. High-stability liquid oils, without the benefit of added antioxidants, and cocoa butter equivalents are two examples of products that can be produced with fractionation technology. Fractionation technologies may also be used to produce base stocks for utilization as components in finished products for various applications (Hastert, 1988).

2.3.5.5 Hydrogenation:

With hydrogenation, cottonseed oil can be converted from a liquid oil into a plastic or solid fat more suitable for numerous applications.

Awide range of edible oils products can be produced with the hydrogenation process, during the hydrogenation process, hydrogen is chemically attached at the double bond sites on the carbon chain of the unsaturated fatty acids. This reaction eliminates a double bond and converts an unsaturated fatty acid to a more saturated fatty acid. Isomerization during the hydrogenation process can also create trans-isomers. Both of these chemical changes increase the melting point of the reacted oil (Hastert, 1988).

Hydrogenation can take place only when the three reactants have been brought together: unsaturated oil, catalyst, and hydrogen gas. The hydrogen gas must be dissolved in the liquid before it can diffuse through the liquid to the solid catalyst surface (Hastert, 1988).

Each absorbed unsaturated fatty acid can then react with a hydrogen atom to complete the saturation to the double bond, shift it to a new position, or twist it to a higher melting trans-form. When the unsaturated oil to be hydrogenated contains mono-, di-, and tri-unsaturated, there is competition for the catalyst surface. The di- and tri-unsaturated are

preferentially absorbed from the oil to the catalyst surface and partially isomerized or hydrogenated to a mono-unsaturated until their concentration is very low, permitting the mono unsaturated to be absorbed and reacted. The variables that can affect the hydrogenation reaction are temperature, agitation, hydrogen pressure, type and quantity of catalysts, feedstock quality, and the fatty acid composition of the source oil (Hastert, 1988).

2.3.5.6 Base stock systems:

Base stock systems with a limited number of hydrogenated stock products for blending to meet the finished product requirements are used by most processors for better control and efficiency. Base stock requirements will vary with each processor, depending on the customer requirements, which dictate the finished products produced. A base stock system an include several source oils, or be limited to a single source oil if that oil's composition can provide all of the properties necessary for the finished product's functionality (Brien, 2003).

Use of this base stock system should enable a processor to produce most formulated edible oil products by blending two or more of the base stocks, except for some specialty products that can only be made with special hydrogenation conditions or a lauric oil. The base stocks represent saturation levels, starting with natural cottonseed oil followed by six hydrogenated base stocks ranging from an iodine value of 80 to almost complete saturation (Brien, 2003).

2.3.5.7 Post bleaching:

A separate bleaching operation immediately after the hydrogenation process has three purposes:

a) To ensure that all traces of the prooxidant hydrogenation catalyst that may have escaped the filtration system after hydrogenation have been captured.
b) To remove undesirable colors that may have been accentuated during hydrogenation.
c) To remove peroxide and secondary oxidation products. Post bleach systems can be exact duplicates of the prebleach process. One difference is that a chelating acid, either citric or phosphoric, should be used to ensure that any residual nickel content is reduced to the lowest possible level(Brien and Wan, 2000).

Batch systems are preferred for post bleaching over continuous for the same reasons as for hydrogenation systems—production of a wide variety of Hydrogenated base stocks (Brien and Wan, 2000).

2.3.5.8 Interesterification:

The least known and practiced processing technique available to the fats and oils processor for modification of the physical properties of an oil is interesterification, often referred to as rearrangement. The ability to modify the melting point and functional crystallization characteristics without changing the fatty acid composition makes interesterification a process with a number of unique possibilities (USDA, 1998).

During the Interesterification process, the triglyceride ester bonds are broken, the resulting fatty acids mix together, and eventually they reattach. If the fatty acids reattach to the same glycerol molecules, the reaction is called intraesterification. When the fatty acids attach to different glycerol molecules, it is called interesterification. A random equilibrium arrangement of the fatty acids is reached if the reaction is allowed to

continue long enough. The primary benefits are modification of the physical properties, such as, melting point and solid fat index values, and modification of the crystal form tendencies. Similar types of physical changes are possible with hydrogenation or fractionation, but both of these processes change the fatty acid distribution of the final product (Norris and Amer, 1947).

The process steps are as follows:

a) Heat the oil at 250°F to 300°F or 120°C to150°C in the reaction vessel under a vacuum to dry the oil. Drying is critical because moisture deactivates the catalyst.
b) After drying, cool the oil to the reaction temperature, suck in the catalyst with the vacuum, and agitate for 30 to 60 minutes or until the distinctive brown color is formed indicating randomization. Analyze the reaction mixture with a refractometer to determine if the reaction is complete or requires additional catalyst and/or time to reach the predetermined analytical endpoint.
c) When reaction completion is confirmed, the catalyst is neutralized either with the addition of phosphoric acid or water washing.
d) The neutralized interesterified oil must be post bleached to remove the brown color formed during the reaction before blending and/or deodorization (Norris and Amer, 1947).

Continuous processes are normally used for directed interesterification because the batch process is difficult to control and a number of reaction vessels would be required. Scraped-wall heat exchangers cool the mixture to initiate crystallization and later remove the heat liberated when the trisaturates crystallize. The trisaturates formed can be separated by filtration, or the reaction can be controlled to produce the trisaturated level

desired for a compound-type shortening formulation (Norris and Amer, 1947).

2.3.5.10 Blending:

Various base stocks are blended to produce the specified composition, consistency, and oxidative stability for edible fats and oils products, such as shortenings, frying fats, margarine oils, specialty products, and even some salad oils. The base stocks may be composed of natural source oils and/or modified oils produced with hydrogenation, fractionation, or interesterification processes (Brien, 1998).

Blends are made to meet both the composition and analytical consistency controls identified by the product developers and quality assurance.

The blending process requires storage tanks to inventory the base stocks and scale tanks and/or meters to proportion the base stocks accurately for each different product. The blend tanks should be equipped with agitators and heating coils to assure a uniform blend (Brien, 1998).

2.3.5.10 Deodorization:

Deodorization is the last processing step, in which flavor and many of the stability qualities of an edible fat and oil product can be controlled. To produce quality deodorized products, attention must be focused on all of the factors involved with the process. The deodorization physical process removes the volatile, odoriferous materials present in the oils.

Many factors that influence the quality of the deodorized oil products are (Brien, 1998).

2.3.5.10.1 Undeodorized oil preparation:

The first process control requirement is to assure that the processing of the oil prior to deodorization has been preformed properly. Preparation of the oil before deodorization has a significant effect on the product after deodorization (Wakelyn, 2003).

2.3.5.10.2 Air elimination:

After deodorization, the oils must be protected from air to preserve the deodorized oil quality. The usual procedure is to replace air with nitrogen. Oxygen contact can be reduced considerably by keeping the entire handling system after deodorization protected with an inert gas like nitrogen (Wakelyn, 2003).

2.3.5.10.3 Metal chelating:

Many of the trace metals promote autoxidation, which results in off-flavors and odors accompanied by color development (Wakelyn, 2003).
The effects of prooxidants can be diminished by using chelating agents before and after deodorization. The most commonly used chelating agents are citric acid, phosphoric acid, and lecithin. Deodorized oils are usually treated with citric acid during the cooling cycle at 50 to 100 ppm (Wakelyn, 2003).

2.3.5.10.4 Oil polishing:

The final stage of deodorization is filtration of the oil to remove any fine particles of soaps, metallic salts, rust, filter aid, polymerized oil, or any other solid impurities (Wakelyn, 2003).

2.3.5.4.5 Antioxidant addition:

Vegetable oils contain tocopherols that are natural antioxidants. Tocopherols are removed in the refining, bleaching, and deodorization processes, but enough survive to provide the optimum stability available from the natural antioxidants. Several phenolic compounds have been identified that can also provide oxidative stability and longer shelf life or fats and oils by delaying the onset of oxidative rancidity. Phenolic substances or antioxidants function as free radical acceptor, thereby terminating oxidation at the initial step. Several antioxidant compounds are available, but tertiary-butylhydroquinone (TBHQ) is the most effective antioxidant for vegetable oils (Wakelyn, 2003).

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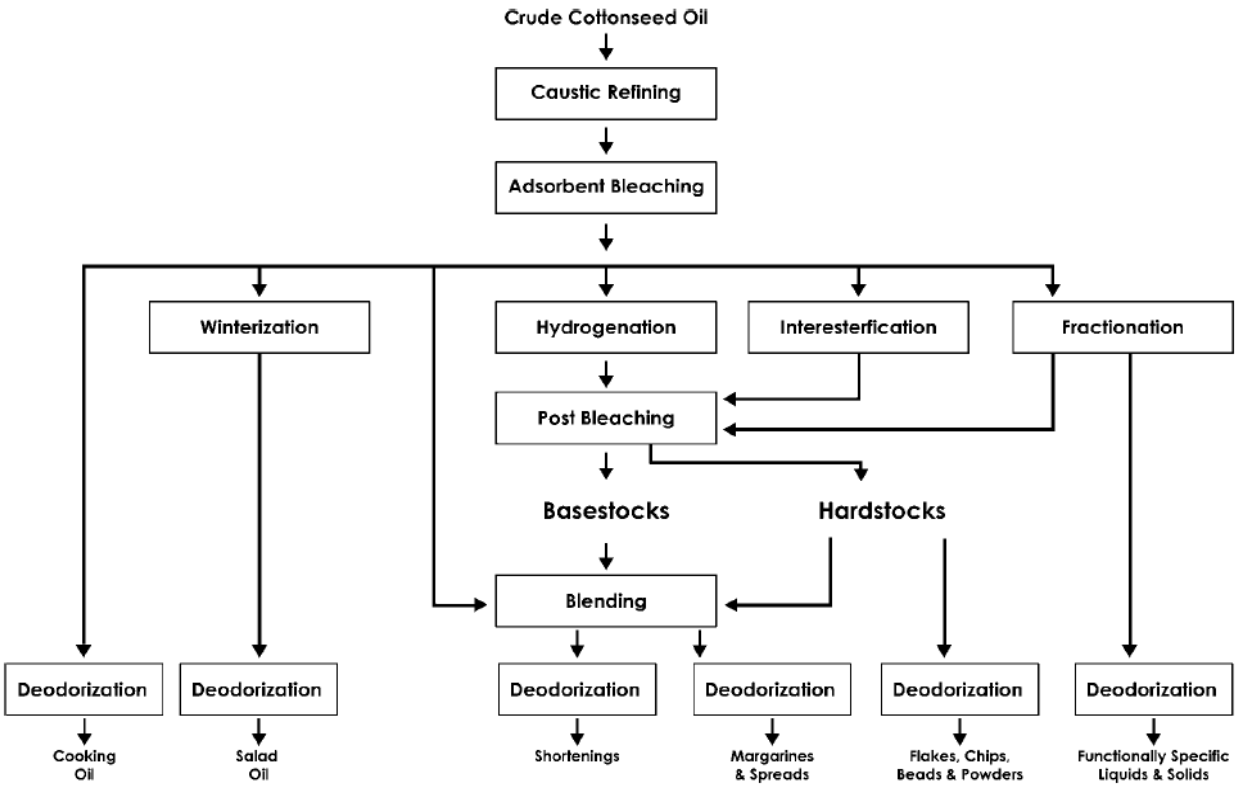

Figure(2): Cottonseed oil processing-flow sequences

2.4 Cottonseed oil utilization

Cottonseed oil is a more universal source oil to provide the desired functionality for most products its used in liquid oils, shortenings, margarines, and specialty products. The United States vegetable oil industry was developed with cottonseed oil as the original source oil, and it dominated this market for almost 100 years. Many of the prepared food products available today were developed with a shortening, margarine, or an oil product containing cottonseed oil Figure (5) (USDA, 2002).

2.4.1 Liquid oils:

Liquid oils are identified by their functionality traits; cooking, salad, and high stability. The definition for each of these classifications is as follows.

2.4.1.1 Cooking oil:

An edible oil that is liquid and clear at room temperature, 75°F or 23.9°C , that may be used for cooking. Cooking oils are typically used for pan frying, deep-fat frying, sauces, gravies, marinates, and othernon-refrigerated food preparations where a clear liquid oil has application. Cooking oils usually congeal or solidify at refrigerator temperatures. Refined, bleached, and deodorized cottonseed oil is considered a cooking oil because it contains saturated fatty acids that cause the oil to cloud and solidify slightly below room temperature (USDA, 1971 and USDA, 1998).

2.4.1.2 Salad oil:

An edible oil that is suitable for the production of a mayonnaise or salad dressing emulsion, which will remain liquid at refrigerated temperatures 40°F or 4.4°C (USDA, 1971 and USDA, 1998).

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2.4.1.3 High stability oils:

An edible oil that is clear at room temperature and possesses an exceptional oxidative and flavor stability.

High-stability oils can be produced by hydrogenation followed by fractionation or by genetic engineering. Most high-stability oils are considered specialty products (USDA, 1971 and USDA, 1998).

2.4.2 Shortenings:

Shortening is an American invention developed with cottonseed oil to replace lard, the solid fat of choice. In 1948, H. C. Black defined shortening as a semisolid plastic material made wholly from fats and oils for use in cooking, baking, and frying (Black,1948).

Initially, shortenings were produced to resemble the consistency and plasticity of lard. Now, shortenings are designed to satisfy individual specific requirements for all of the food industry as well as offering products with broad general appeal. With this broader application, shortening consistency varies from wide workable ranges to brittle products with sharp melting characteristics from very firm consistencies to liquid or pumpable products, or from creamy, smooth textures to grainy structures depending on the requirements of the application (Brien, 2000, Brien1998, Bell, 1991).

Adescription for shortening would be: processed edible fats and oils that affect flavor, oxidative stability, shelf life quality, eating characteristics, nutrition, and the eye appeal of prepared foods by providing emulsification, lubricity, structure, aeration, moisture barrier, flavor medium, or heat transfer (Brien, 2000, Brien1998, Bell, 1991).

Shortening product are prepared with various blends of many different oil under diverse processing, conditions. The processing, conditions, ratio of solid to liquids, nitrogen amount, Crystalline Properties, and other factors must be considered in order to achieve the desired functionality and nutritional quality (Brien and Hui, 1996).

2.4.3 Margarine and spreads:

Margarine is a flavored food product containing 80% fat, made by blending selected fats and oils with other ingredients, fortified with Vitamin A, to produce a table, cooking, or baking fat product that serves the purpose of dairy butter but is different in composition and can be varied for different applications (Brien and Hui, 1996).

Margarine was developed to fill both an economic and a nutritional need when it was first made as a butter substitute. There are over ten different types of margarine produced today, including regular, whipped, soft tub, liquid, diet, spreads, no fat, restaurant, bakers, and specialty types, which are packaged in as many different packages. These margarines are made from a variety of fats and oils such as cottonseed.

Margarine products cater to the requirements of all the different consumers; retail, food service, and food processor (Brien, 1998).

2.4.4 Other cottonseed oil uses:

Food applications have been a major use for cottonseed oil but it has also been used in soap, lubricants, sulfonated oil, pharmaceuticals, protective coastings, rubber, as a carrier for nickel catalysts, and, to a lesser degree, in the manufacture of leather, textiles, printing ink, polishes, synthetic plastics, and resins (Bailey,1948).

2.5 Characteristics of oil:

Whole cottonseed contains from (17-23) percent oil which has a high content of linoleic acid (46-24%) variable percentage of oleic acid (18-24%) of palmetic acid .The other fatty acids (myristic, stearic, arachic, myristoleic, palmitoleic) are present in very small percentage (from 0.2 to 2% each), iodine value of cottonseed oil is in the range (99-113), while the saponification value ranges between (189-198) (Stansbury *et al*.,1954).

2.6 Cottonseed oil properties:

Crude cottonseed oil has a strong, characteristic flavor and a dark, reddish brown color from the presence of highly colored material extracted from the seed. It is a member of a particularly useful group of vegetable oils whose fatty acids consist substantially of 16 and 18 carbon fatty acids containing no more than two double bonds. Cottonseed oil is stable in the beta-prime crystal form, which is desirable in most solidified products because it promotes a smooth, workable consistency usually referred to as plasticity (Stansbury, 1954).

A number of factors are responsible for minor variations in the composition of cottonseed oil before it is extracted from the seed. These include climate, growing region, variety of cotton grown, the agronomic practices employed, and the handling/storage of the cottonseed before crushing. Due to the interaction of all these factors in any one sample of oil, it is difficult to make clear generalizations about quality variations (Stansbury, 1954).

2.6.1 Cottonseed oil glyceride composition:

Chemically, all fats and oils are esters of glycerol and fatty acids; nevertheless, the physical properties of natural fats and oils vary widely, this is because:

a) The proportion of the fatty acids vary over wide ranges .
b) the triglyceride structures vary for each individual oil and fat. Fats and oils are commonly referred to as triglycerides because the glycerin molecule has three separate points where a fatty acid can be attached. All triglycerides have identical glycerol components that leave the fatty acids to contribute the different properties (Wan *et al*., 2000).

For all practical purposes, contain fatty acids with carbon chain lengths between 4 and 24 carbon atoms with zero to three double bonds (Wan *et al*., 2000).

2.6.1.1 Saturated fatty acids:

which contain only single carbon-to-carbon bonds, are chemically the least reactive and have a higher melting point than corresponding fatty acids of the same chain length with one or more double bonds. Most of the natural saturated fatty acids have an unbranched structure with an even number of carbon atoms. the most important saturated fatty acids are butyric (C4:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic (C22:0), and lignoceric (C24:0). The melting point of saturated fatty acids increases with chain length. Saturated fatty acids with 10 and longer carbon chains are solids at room temperature (Wan *et al*., 2000).

2.6.1.2 Unsaturated fatty acids:

which contain one or more carbon-to-carbon double bonds, are the most chemically reactive and those with the most double bonds are the most reactive. A fatty acid containing only one double bond is called monounsaturated; the most notable is oleic (C18:1). When a fatty acid contains more than one double bond, it is identified as polyunsaturated. The notable polyunsaturated fatty acids are the essential fatty acids linoleic (C18:2) and linolenic (C18:3). These fatty acids are essential in the sense that the human body needs them and yet cannot either synthesize at all or in sufficient quantities. In nature, the double bonds are cis-form, which has both hydrogen atoms on the same side of the double bond. Trans-form fatty acids, with the hydrogen atoms on opposite sides of the double bond, are thermodynamically more stable. Trans-fatty acids are cis-form fatty acids that have been isomerized by oxidation or hydrogenation (Wan *et al*., 2000).

2.6.2 Cottonseed oil fatty acid composition:

The specific fatty acid profile of the triglycerides in cottonseed is dependent on the variety of cotton grown, growing conditions such as temperature and rainfall, and the analytical method used to determine the profile. Cottonseed oil is typical of the oleic–linoleic group of vegetable oils, because those two fatty acids comprise almost 75% of the total fatty acids. Although oleic acid makes up 22% and linoleic makes up 52%, less than 1% linolenic acid is present. Palmitic fatty acid makes up about 24% of the fatty acids. Minor amounts of other saturated fatty acids are also found. The composition of American cottonseed oils will rarely fall outside of these limits: 23–28% total saturated fatty acids, 22–28% oleic acid, and 44–53% linoleic acid (Bailey, 1948).

2.6.3 Cottonseed oil triglyceride composition:

The triglyceride structure of an edible fat or oil is affected by which carbon atom of the glycerol has the fatty acid linked, whether the three fatty acids are the same or different, and the position of each. Triglycerides with three identical fatty acids are called simple or monoacid triglycerides. Triglycerides containing more than one type of fatty acid are called complex or mixed triglycerides. A mixed triglyceride containing three different fatty acids has three isomeric forms, depending on which fatty acid is in the middle, 2 or beta position of the glycerol portion of the molecule and which fatty acids are in the alpha or outside positions. Therefore, both the chemical and physical properties of fats and oils are largely determined by the fatty acids that they contain and their position within the triglyceride structure. As linoleic, oleic and palmitic fatty acid account for over 90% of the fatty acids in cottonseed oil, most of the triglycerides contain some combination of these fatty acids (Jurriens *et al*, 1965).

The predominant pair includes palmitic acid as the saturated acid in acyl positions 1 and 3, whereas position 2 is occupied by oleic or linoleic acid).The amounts and the types of fatty acids and the interpositional and intrapositional distribution result in various triglyceride forms that contribute to the various functional properties of cottonseed oil (Jurriens *et al*, 1965) .

2.6.4 Cyclopropenoid fatty acids:

Cotton contain a pair of unique cyclopropene fatty acids (CPFA). These two fatty acids, sterculic and malvalic acid, are generally referred to collectively as cyclopropenoid fatty acids. Sterculic acid is the most active of the two fatty acids whose general action is to inhibit the desaturation of

stearic to oleic fatty acid in the animal body with a resultant alteration in membrane permeability or an increase in the melting point of fats(Phelps *et al*., 1965, Carter and Frampton, 1964).

Sterculic and malvalic acids are 18 and 17 carbons long, respectively, and include one double bond at the site of the propene ring, either at the 9,10 position or 8,9 position. The cyclopropene ring is the physiologically active entity of the two fatty acids (Phelps *et al*., 1965, Carter and Frampton, 1964).

The physiological activity of sterculic acid is greater than that of malvalic acid (Allen *et a.*, 1967).

The ratio of malvalic acid to sterculic acid in cottonseed oil is usually about 3 to 1. The cyclopropenyl structure is highly strained, which apparently accounts for its reactivity (Carter and Frampton, 1964).

In cottonseed oil, the CPFA are reduced in processing with the result that cottonseed oil in commercial channels contains a negligible level.

Deodorization is a processing steps responsible forthe greatest inactivation of CPFA. (Phelps *et al*, 1965)**.**

2.7 Cottonseed oil physical characteristics:

The physical properties for all fats and oils, including cottonseed oil, are determined by their individual chemical compositions. Physical properties are of practical importance because most applications depend on the melting behavior, solubility, flavor, density, appearance, and the other physical properties to provide functionality for finished products. Analytical and physical evaluation methods are used to measure these attributes for identification, trading, and control purposes (Illinois, 1999).

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2.7.1 Melting point:

The usual definition for melting point is the temperature at which a material changes from a solid to a liquid. (Illinois, 1999).

The Determination of The melting point is difficult because most fats and oils have aheterogenous composition and the different components melt at different temperatures. The melting point of cottonseed oil is 10-15.6 °C (50- 60°F).The titer is the temperature at which the separetd fatty acids solidify. Refined, bleached, and deodorizd cottonseed oil has arelatively high titer due to its saturated fatty acid content. while winterized oil has a lower titer. the enthalpies and specific heat of cottonseed oil and its fractions have found to be quite similar 25-110°C, The specific of cottonseed oil and its fractions in the range of 25-110°C is 2.4 j/g/k (Kapseu *et al*, 1991).

2.7.2 Solid fat index:

This analysis has become the most important criterion for the melting behavior and crystalline structure of fats and oils products. It determines the proportion of solid and liquid materials at a given temperature. The solid fat index (SFI) analysis is an empirical measure of the solid fat content. It is calculated from the specific volume at various temperatures using a dilatometric scale graduated in units of milliliters times 1000. Values for the solid contents are usually determined at 50°F, 70°F, 80°F,

92°F, and 104°F or 10°C, 21.1°C, 26.7°C, 33.3°C, and 40°C. Unlike the tropical oils, cottonseed and the other oleic- and linoleic- classification oils do not contain any significant quantity of triglycerides made up of two or three saturated fatty acids; therefore, the solid fat index at the lowest temperature usually measured would have minimal values. Natural cottonseed oil can have a solid fat index content at 50°F or 10°C but not at the higher temperature measurements (Illinois,1999).

2.7.3 Refractive index:

The refractive index of fats and oils is an important characteristic because of the ease and speed with which it can be determined precisely, the small amount of sample required, and its relationship to structure. It is useful for source oil identification, for observing progress of reactions rapidly, and for establishing purity value (Swern, 1964).

Refractometers equipped with temperature controls are used for fats and oils. The measurements are usually made at 25°C for soft oils, and the higher melting point products require temperature adjustments to 40°C or 60°C, depending on the decrease as the temperature melting point of the product. The refractive index values is increased but still increases with the length of the carbon chains and the number of double bonds present in the sample. By reference to a predetermined curve relating the refractive index at temperature measured to iodine value, a rapid estimation of the iodine value may be made. One source of error in this method is that trans-acids formed during hydrogenation affect refractive index values but not iodine value (Swern, 1964).

2.7.4 Flash and fire point:

The Flash point is the temperature at which sufficient amount of volatile products are evolved to ignited if an external spark or flame are provided.At the fire point, the accumulated breakdown products are capable of supporting a flame on their own. The flash point of cottonseed oil is generally about 343.4°C, 650 °Fand the fire point about 375.3 °C, 675 °F (Morgan, 1942).

2.7.5 Color:

Crude cottonseed oil has a dark reddish-brown color because of the presence of highly colored material extracted from the seed. After processing, it typically has a rich golden yellow color that is lighter than peanut and corn oils but darker than soybean, sunflower, canola, and safflower oils. Normal processing of prime quality cottonseed oil will yield a finished oil with a color of 2.0-2.5 red as a measured by the Lovibond method. Most of the red color found in cottonseed oil comes from carotene, but most of the color is caused by a minimal residual level of gossypol and gossypol derivatives. The color removal level is dependent on the cottonseed handling and storage conditions prior to extraction (Hron *et al*., 1992).

2.7.6 Flavor:

The flavor of an edible oil product should be completely bland, so that it can enhance the food product's flavor rather than contribute its own.

Cottonseed oil is well known for its initial bland flavor and the nutty flavor it develops with oxidation. It has been used as the standard for comparison with other oils for both flavor and odor. The nutty flavor developed with oxidation is more pleasant than the oxidized flavor of some

of the other oils in the oleic linoleic classifications. Hydrolysis is major cause of off-flavors in food oils. The free fatty acids liberated with hydrolysis have a distinct flavor and odor that are more disagreeable when the fatty acid chain length is shorter than 14 carbons. Cottonseed oil that contains mostly C-16 and C-18 fatty acids does not become unpalatable until the free fatty acids exceed 1.0% (Bailey, 1948)

2.7.7 Viscosity:

The ability of an oil to resist flow is its viscosity. The viscosity of an oil decreases as it temperature rises, saturation and larger molecules such as along chain fatty acid or polymerized thermal breakdown product tend to increased viscosity. The viscosity value of cottonseed oil with specific gravity of 0.9187 and an acid value of 14.24 is 35.88°c at 73.8°c (70.04°F) (Swern, 1979).

2.7.8 Specific gravity:

The specific gravity is the ratio of the weight of a volume of an oil to the weight of an equal volume of water at the same temperature. At 25°C. The specific gravity of cottonseed salad oil is about 0.917. a liter of cottonseed oil with a specific gravity of 0.914 will weigh 914 g or 914 kg/m3. A U.S. gallon of cottonseed oil with this specific gravity would weigh 7.63 lbs (Bailey, 1948).

2.8 Cottonseed oil chemical characteristics:

Cottonseed oil, like all oils and fats, is made up of glyceridic materials with some nonglyceridic material in lessor quantities. It is the chemical composition that defines the chemical and physical properties of all fats

and oils, which in turn will determine the suitability of the oil in various processes and applications.

2.8.1 Free fatty acid:

Oil chemists learned that the free fatty acid (FFA) content was a good indicator of crude cottonseed oil quality in the 1880s.

Crude oil from the best quality cottonseed will have an FFA content of 0.5% to 0.6%. During a good season, the FFA content will be less than 1.0%; however, during unfavorable climatic conditions, the FFA content may average 5% or higher. Dry weather during cotton picking favors low FFA development. Extremely poor oil may contain 15% to 25% FFA. Refining process neutralizes or removes free fatty acids to a level of 0.05%; For chemical refining, the quantity of sodium hydroxide for neutralization is based on the FFA level of the crude cottonseed oil. Refining losses as low as 2.5% to 3.0% are encountered with cottonseed oils containing 0.5% to 0.6% FFA, but oils with high free fatty acid contents may have refining losses as high as 40% to 50% (Bailey, 1948).

2.8.2 Anisidine value:

Anisidine value is a measure of secondary oxidation or the past history of an oil. It is useful in determining the quality of crude oils and the efficiency of processing procedures, but it is not suitable for the detection of oil oxidation or the evaluation of an oil that has been hydrogenated. AOCS Method Cd 18-90 has been standardized for anisidine value analysis (Illinois,1999). The analysis is based on the color reaction of anisidine and unsaturated aldehydes. An anisidine value of less than ten has been recommended for oils upon receipt and after processing (Eskin *et al*., 1996).

2.8.3 Inherent oxidative stability:

The unsaturated fatty acids in all fats and oils are subject to oxidation, a chemical reaction that occurs with exposure to air. The eventual result is the development of an objectionable flavor and odor. The double bonds contained in the unsaturated fatty acids are the sites of this chemical activity. An oil's oxidation rate is roughly proportional to the degree of unsaturation; for example, linolenic fatty acid (C18:3), with three double bonds, is more susceptible to oxidation than linoleic (C18:2), with only two double bonds, but it is ten times as susceptible as oleic (C18:1), with only one double bond.

Oxidation deterioration results in the formation of hydroperoxides, which decompose into carbonyls, dimerization products, and polymerized gums. It is accelerated by temperature, oxygen pressure, prior oxidation, metal ions, lipoxidases, hematin compounds, antioxidant reductions, absence of metal deactivators, time, and ultraviolet or visible light. Extensive oxidation will eventually destroy the beneficial components contained in many fats and oils, such as the carotenoids or vitamin A, the essential fatty acids (linoleic and linolenic), and the tocopherols or Vitamin E. Oxidative stability estimates can be made from the iodine value measurement, calculated iodine value from the fatty acid composition, or an oxidative stability formula (Erickson and List., 1985).

2.8.4 Thiocyanogen value:

This test also indicates the degree of unsaturation its considered to be less reliable than (IV). The (SCN2) used in this test does not to the double bonds of linoleic and linolenic in the same manner as iodine, thus the values are different. Typical Thiocyanogen values for cottonseed oil are 60-72 (Bailey, 1948).

2.8.5 Iodine value:

Iodine value (IV) is a simple and rapidly determined chemical constant that measures the unsaturation of an oil, but it does not define the specific fatty acids. The iodine value procedure determines the grams of iodine absorbed by 100 g of oil. A higher iodine value indicates a greater number of double bonds. The iodine value results for cottonseed oil vary somewhat from year to year, sections of the country, and by growing season. A cooler growing season provides oil with a higher than average linoleic fatty acid (C18:2) content with a lower oleic fatty acid (C-18:1) content; warmer growing seasons reverse this trend. These variations increase or decrease the number of double bonds, which affects the iodine value. Typically, cottonseed oil iodine values range from 103 in Texas to 112 in other regions of the United States (Brien, 1998).

2.8.6 Halphen reaction:

The halphen test is a very sensitive and reliable method for detecting the presence of cottonseed oil in another oil. A reaction with sulfur in carbon disulfide mixed with equal amounts of amyl alcohol gives a cherry red color when cyclopropenoid fatty acids unique to the Malvacae family, which includes cottonseed and okra, are present. This test is capable of detecting 0.25% or less cottonseed oil in an oil blend. The oil is no longer responsive to the halphen test after hydrogenation, which decreases the iodine value 2–5 units(Brien, 1998).

2.8.7 Unsaponifiable matter:

Unsaponifiable matter are those substances dissolved in an oil that cannot be saponified by alkalies but are soluble in nonpolar solvents.

These materials are made up of sterols, hydrocarbons, tocopherols, pigments, and higher materials that are insoluble in water. The level of unsaponifiable matter in good-quality cottonseed oil usually ranges from 0.5% to 0.7% with slight decreases after refining and deodorization. (Bailey, 1948).

2.8.8 Saponification value:

Potassium hydroxide is added to saponify 1.0g of the oil. The weight in milligrams of KOH used is inversely related to the molecular weight of the fatty acid in the oil . Cottonseed oil saponification values range from 189 to 198 with an average of 195 (Weiss, 1983).

2.9 Cottonseed oil nonglyceride components:

Cottonseed oil is unusual for the amount and variety of nonoil substances in the crude oil. Its content of nonglyceride substances, exclusive of free fatty acids, commonly amounts to 2% or more in the crude state that contribute to the strong red brown colour and odor of the unrefined oil. These minor components, identified as the unsaponifiable fraction, consist of gossypol, phospholipids, tocopherols, sterols, resins, carbohydrates, pesticides and other pigments (Marchlewski, 1899)

2.9.1 Gossypol:

2.9.1.1 History of gossypol:

As early as 1861 Kuhlmann found a yellow pigment in the cotton plant with properties which lead us to think it the same as the substance later isolated by (Marchlewski, 1899) and given name gossypol.Kuhlmann attempted to recover this substance from the "foots" of cottonseed oil after removal of the fatty acids. He obtained an impure product having a

greenishblue color, characteristic of the oxidation products of pure gossypol. (Longmore, 1866) in an attempt to isolate the coloring mat*ter* of crude cottonseed oil obtained a brown powder which he described as having a pungent powerful dyeing properties. This substance was also quite impure, and likely a mixture of the decomposition products of gossypol rather than a single compound. The work of Marchlewski who gave the compound its name by a combination of the two words gossypium and phenol, seems to have led the way for the more recent investigations although very little more was published on the subject until the notable work of (Withers and Carruth 1915).

(Carruth's and Frank, 1918) translation of Marchlewski's method of obtaining gossypol gives some indication of the difficulties involved in obtaining pure product without oxidation and possibly the occurrence of molecular rearrangements. Carruth states that gossypol ceappears to be a constitutent of the cotton plant only. He goes on to say, lt occurs, in peculiar glands called 'gland dots, 'secretion glands' or 'resin glands' which are present in all parts of the plant except the woody tissue. These are 100 to 400 m. in diameter and are readily visible to the eye. They appear to be formed by disintegration of adjacent cells.

All of these glands as well as those found in the seeds, Source of material which we have been using for the isolation of gossypol, give a characteristic red color with sulphuric acid which suggests the presence of gossypol Schwartze and Aiseberg (1923) found a correlation between the gossypol and the locality in which they grew.

2.9.1.2 Definition of gossypol:

Gossypol is a toxic compound found in the cotton plant. It is concentrated in the cottonseed but can also be found in other parts of the

cotton plant such as hulls, leaves, and stem (Morgan, 1989).Gossypol is polyphenolic yellow pigment found naturally throughout the cottonplant (Cheery, 1978) it is found in both vegetative and reproductive tissues of the cotton plant. The pigment is located almost exclusively in pigment gland structures appearing as dark dots in the plant tissue. The absence of pigment glands does not ensure elimination of gossypol, (Fisher *et al*., 1988).

The glands in cottonseed contain predominantly gossypol with traces of desoxyhemigossypol, while glands in the foliage contain hemigossypolone and heliocides (Benedict *et al*., 2004). Gossypol is the primary constituent of these glands, and its concentration in cotton foliage and seed is directly related to gland density (Lukefahr *et al*., 1966).

Gossypol protect the plant from pests and possibly some diseases (Bottger *et al.*, 1964, Bell and Stipanovic, 1977, Hedin *et al.*, 1992, Jenkins and Wilson, 1996).Gossypol has also been shown to have *invitro* and *invivo* inhibitory activities against diverse pathogenic agents, such as *Tryeanosoma cruzi*(Montamat *et al.*, 1982, Abe *et al.*, 2004)*Plasmodium falciparum*(Royer *et al*., 1986; Tripathi *et al*., 2004); has anti-tumor activity (Blackstaffe *et al.*, 1997); as well as contraceptive (Maltin, 1994).

This gossypol significantly drew the attention because of it is toxicity and also for being localized in the seeds having nutritional value, i.e high oil and protein contents (Moore and Rollins, 1974).

2.9.1.3 Chemical structure of gossypol:

Gossypolis atriterpenoid aldehyde (Figure3) it is l,1,6,6'hexahydroxy-5,5'-diisopropyl-3,3'dimethyl (2,2'-binaphthalenel)-8,8'-dicarboxaldehyde, is a 30-carbon, 4-ring structures ($C_{30}H_{30}O_8$) (Berardi and Goldblatt 1969; Randel *et al*., 1992). The structure of gossypol comprises two modified

naphthalene rings connected at 2,2 position. Three functional groups attached to the rings, those are; two aldehyde groups at the 8.8 position; six hydroxyl groups at the 1.1.6,6,7,7 posions; and four alkyl groups; two methyl at 3,3 positions and two isopropyl groups at the 5,5 positions (Abou-Donia, 1976) .

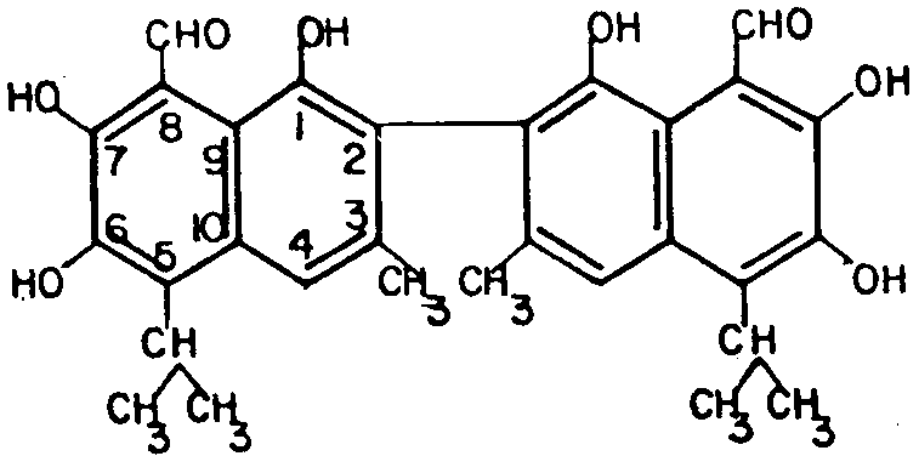


Figure (3):Structure of gossypol (1.6.6 ,7,7 hexahydroxy-3.3.dimethyl-5,5, diisopropyl-2,2,.binaphythyl-8,8 dialdehyde).

2.9.1.4 Chemical properties:

The compound is yellow, soluble in organic solvents, and maximum absorption wavelength 385 nm and molar extinction coefficient 18,000 in ethanol (Tso and Lee , 1982). Gossypol is relatively stable when dissolved in organic solvents and stored in the dark at -20^0C, with a molecular weight of 518.54. Gossypol is composed of three tautomeric forms that account for the numerous chemical and physiological reactions often associated with this compound (Berardi *et al*., 1969; Randel *et al.*, 1992).

2.9.1.5 Physiological function:

Gossypol occurs in two forms: free gossypol (FG) and bound gossypol (BG) and the first is "physiologically active". During seed preparation, cooking and hot pressing to obtain edible oil, the heat and moisture cause binding of the aldehyde group to carbohydrate, phospholipids and alpha and omega amino acids to produce so-called "biologically inactive" bound gossypol. This binding causes nutritional proplems to monogastric animals. The effect of free gossypol ranges from toxicity to actual death when those animals are fed cottonseed meal containing high level gossypol. Also discolored egg yolk, and deleterious effects on hatchability, when those eggs are used for reproductive purpose (Berardi and Goldblatt, 1980).

2.9.1.6 Gossypol in cottonseed:

The amount of gossypol in cottonseed is dependent on environmental factors (Cheery et al., 1978) and species of cotton (Boatner *et al*., 1949). Gossypol exists in cotton plant as two stereoisomers, or mirror images of each other, which are designated as (+) and (-) isomers. These two isomers exist in cottonseed in two distinct states: bound and unbound.,the unbound form of this compound has been shown to be most biologically active in the animal, the bound gossypol is essentially unavailable to the animal, (because these are chemical determinations) but the possibility for some crossover of biological activity exists (Calhoun et al., 1995a).Most gossypol found in whole cottonseeds (WCS) is in the free form, but some becomes bound due to heat, moisture and pressure associated with CS meal extrusion, and other types of CS processing (Mena *et al.*, 2001; Calhoun *et al*., 1995a).Gossypol also exists as a mixture of (+) and (−) stereoisomers,with the (−) isomer having the higher biological activity (Joseph *et al.*, 1986, Lordelo *et al*., 2005, Yu, 1987). The ratio of (+) to (-)

isomers in Upland cottonseed(*Gossypium hirsutum*) was found to be 56.6% to 43.4%, respectively while Pima cottonseed is 48.8% to 51.2%, respectively of gossypol (Sullivan et al., 1993a).

Cottonseed has been shown to contain from 0.40 to 2.0% free gossypol. (Boatner and Charlotte, 1948; Berardi and Goldblatt, 1969). Whole cottonseed typically contains 1.5-2.0% gossypol, all in the unbound form (Calhoun 1995a,Nomeir and Abou-Donia, 1985).Hulls typically reported as having less than 0.049% free gossypol content(Foster and Calhoun, 1995). The presence of gossypol affords the plant some protection against predators such as insects, field mice, and raccoons that might otherwise feed on these plants and/or their seeds (Boatner and Charlotte, 1948; Berardi and Goldblatt, 1969).

Following removal of the fiber, the seeds undergo further processing to remove the oil, either by crushing or solvent extraction. The remaining meal is used as a feed material because of the high protein content, particularly for ruminants that are less sensitive to gossypol than other species (Adams et al., 1960; Markman and Rzhekhin,1969; Jaroszewski, 1998; Dodou, 2005).

2.9.1.7 Mechanisms of toxicity:

In several studies the mechanisms of toxicity of gossypol have been explored (Ali and El-Sewedy, 1984; Tso and Lee, 1982; Gawai *et al.*, 1995; Kovacic, 2003; Fiorini., 2004; Zhou., 2008). Gossypol and its metabolites exert pro- and anti-oxidant potential.

Considerable evidence points to oxidative stress, formation of reactive oxygen species, and DNA scission, characteristics of redox-cycling by electron transfer in biosystems (Kovacic, 2003). In rats treated intraperitoneally for 5 days with 5 mg/kg b.w. of racemic gossypol perday

biochemical changes in liver enzymes were observed with a decrease in cytochrome P-450, cytochrome b5,NADPH-cytochrome reductase, aminopyrineN-demethylase and aniline hydroxylase. However, the treatment did not affect levels of cytosolic glutathione Stransferase (GST) and the serum enzymes sorbitol dehydrogenase and alanine aminotransferase, which are indicators of liver damage (Ali and El-Sewedy, 1984). With respect to GST isozymes, gossypol is areversible inhibitor (Lee *et al.*, 1982). Furthermore, gossypol binds to microsomal membranes, inhibits DNA synthesis, and causes depletion of iron and glutathione in mammalian cells (Gawai *et al.*, 1995).

Gossypol also specifically inhibits the 11β-hydroxy-steroid dehydrogenase (11β-HSD), which oxidises cortisol to inactive cortisone in the kidney and other tissues of importance in electrolyte potassium and sodium regulation. Episodes of hypokalemia observed in clinical trials for antispermatogenic effect are the clinical consequence of inhibition of 11β-HSD (Song *et al.*, 1992; Waites *et al.*, 1998; Reidenberg, 2000).

Another mechanism relevant to the anti-fertility effects of gossypol is the ability to block gap junction intercellular communication (GJIC) in Sertoli cells, required for spermatogenesis, which has been demonstrated in cultured human and rat cells (Herve *et al.*, 1996). *In vitro* experiments in Sertoli cells showed a rapid cytoplasmic delocalization of connexin 43 (a gapjunctional protein important for Sertoli cells to regulate spermatogenesis) In addition Ncadherin and connexin 43 protein expressions were decreased (Fiorini *et al.*, 2004; Zhou *etal.*, 2008).

More recently, racemic gossypol has been shown to induce apoptosis and inhibit *in vitro* proliferation of cancer cell types (Dunning, prostate, epithelial (breast), stromal, cervical, colon). These inhibitory effects have been associated with the induction of TGFβ1 in humanprostate cancer PC3

cells which in turn influences the expression of the cell cycle-regulatoryprotein (Jiang *et al.*, 2004; Liu., 2005).

Finally, gossypol scavenges free radicals, reduces ferric ions, prevents UV-induceddeoxyribonucleic acid (DNA) damage and inhibits the growth of *Trypanosoma brucei* cells (Wang *et al.*, 2008).

2.9.1.8 Toxicity to human:

Gossypol is localized in those pigment glands and used in medicine because of its anti-tumor and anti-cancerogen properties and provides endurance against plant mites, fungus and bacteria. It also has antifertility properties in males (Cass *et al*., 1991; Vroh Bi *et al*., 1999; Benson *et al*., 2001; Coutinho, 2002; Qiu *et al*., 2002; Cai *et al*., 2004; Meyer *et al*., 2004; Sotelo *et al*., 2005; Kline *et al*., 2008; Moon *et al*., 2008). The main function of gossypol in the cotton is to act as a natural insecticide (Berardi and Goldblatt, 1980)

In 1957, a village in the Jiangsu province in China did not have any childbirth between the 1930s and 1940s. However, the villagers were fecund before and after that period. This infertility incident was due to a large-scale contamination of cotton oil for human consumption with gossypol (Qian and Wang, 1984; Amini and Kamkar, 2005). Furthermore, in the 1960s, farmers from the Hubei and Hebei provinces in China that ingested home made unheated cottonseed oil developed fatigue and a burning sensation on the face and other exposed parts of the body and these symptoms were qualified as “Hanchuan fever or burning fever” (Wu, 1989). Hence, gossypol attracted a lot of attention as a possible male antifertility agent or a therapeutic agent for some gynaecological diseases, but the research in this area was discontinued due to irreversibility of its

anti-spermatogenic effect already at low doses (Dodou, 2005; Waites *et al*., 1998).

Large scale studies involving more than 8000 Chinese males on the use of gossypol as an anti-contraceptive have been carried out using 20 mg (±)-gossypol/day and the study revealed that the drug was efficient and well tolerated, and did not cause changes in blood pressure or biochemical parameters. However, a side effect (hypokalemia) affected around 10 % of Chinese users (National Coordinating Group on Male Antifertility Agent, 1978; Liu., 1985; Coutinho, 2002).

Further trials tested gossypol as a contraceptive agent for men at lower doses enrolling 151 men from various ethnic origins (Brazil, Nigeria, Kenya and China) which received 15 mg racemic gossypol/day (0.24 mg/kg b.w. per day) for 12 or 16 weeks and 51 control subjects. Subjects were then randomized to either 7.5 or 10 mg/day corresponding to 0.12 and 0.17 mg/kg b.w. per day for further 40 weeks. Spermatogenesis suppression was attained in 81 of the 151 treated subjects and only one subject discontinued treatment (because of tiredness). Potassium levels fluctuated within the normal range, follicle stimulating hormone (FSH) levels increased consistently (indicative of reduced secretion of inhibin from Sertoli cells in the testes), testicular volume decreased, but after discontinuation values returned to control levels. Fifty one percent of the subjects who received 0.12 and 0.17 mg/kg b.w. per day recovered sperm counts to 20 million/mL within 12 months of discontinuing gossypol treatment. However, 18 percent of the remaining 48 patients were still azoospermic one year after termination of gossypol treatment. All men diagnosed with varicocele failed to reverse spermatogenesis suppression. Gossypol blood levels indicated that sperm suppression occurs independently of concentration, whereas spermatogenesis recovery appears

to be concentration-dependent. In a study by (Kannedy *et al*., 1983), human semen exposed to gossypol was found to be irreversibly inhibited in the conversion of proacrosin to acrosin. The investigators also found a parallel decline in oocyte penetration; however, a decrease in sperm motility, or forward progression, was not found. This is in conflict with results found by Cowart *et al.* (1994), in which exposure of human semen to gossypol inhibited sperm motility in both a dose- and time-dependent manner by a CAMP-dependent mechanism. The investigators found that this effect was in fact partially reversible by increasing the amount of CAMP.

2.9.1.9 Toxicity to animal:

Gossypol exhibits a variety of biological actions, which range from apparently highly specific pharmacological activities on certain macromolecular targets such as enzymes involved in maturation of mammalian sperm to more unspecific binding to proteins.

Signs of gossypol toxicity are similar in all animals and include laboured breathing and anorexia. Acute toxicity has been shown in the heart, lung, liver, and blood cells resulting in increased erythrocyte fragility. Post mortem findings include generalised oedema and congestion of lungs and liver, fluid-filled thoracic and peritoneal cavities, and degeneration of heart fibers. Reproductive toxicity is seen particularly in males, where gossypol affects sperm motility, inhibits spermatogenesis and depresses sperm counts, cause Sertoli cell toxicity and may also affect Leydig cells. Gossypol also seems to disrupt oestrus cycles, pregnancy and early embryo development, particularly in the monogastric species studied (Abou-Donia, 1976; Berardi and Goldblatt, 1980; Randel *et al.*, 1992; Dodou, 2005). Acute gossypol intoxication of non-ruminant animals causes circulatory

failure. Single-dose oral LD50 of racemic gossypol is 2400-3340 mg/kg for rats, 500-950 mg/kg for mice, 350-600 mg/kg for rabbit, 550 mg/kg for pigs and 280-300 mg/kg for guinea pigs. Sub-acute toxicity causes pulmonary oedema and symptoms of malnutrition. Dogs appear to be quite sensitive (repeatedoral dose of 10-200 mg/kg for a month was fatal). Compared with rodents, ruminants are less susceptible to cottonseed related toxicity (Abou-Donia, 1976). Gossypol is particularly toxic to swine, while poultry and horses seem to be relatively unaffected (Berardi and Goldblatt, 1969). It also has a detrimental effects on monogastric animals and anti-nutritional effects on warm-blooded animals fed cottonseed products (Eisele 1986, Blom *et al*., 2001, Henry *et al*., 2001). It is however, even less toxic to adult ruminant animals because the rumens have the ability to bind large amount of previously free gossypol with soluble proteins, thus inactivating it (Haschek *et al*., 1989, Kim *et al*., 1996, Santos *et al*., 2003). However, young calves are highly susceptible to gossypol toxicity before the rumen is fully functional (Berardi and Goldblatt, 1969).

The two enantiomers of gossypol have markedly different biological effects. In comparis on with (+)-gossypol, the (–)-enantiomer generally exhibits more pronounced effects. For example, (–)-gossypol is more cytotoxic (Band *et al*., 1989; Benz *et al*., 1990; Blackstaffe *etal*., 1997; Shelley *et al*., 1999), binds more strongly to proteins (Wang *et al*., 1992; Oliver, 2005), is the active anti-spermatogenic agent, and is considered more toxic than the (+)- gossypol (Matlin et al., 1985; Lindberg et al., 1987; Bailey et al., 2000; Lordelo et al., 2005)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Samples:

Cottonseed of four different varieties were obtained from The Agricultural Research Corporation (ARC), Wad Medani.

3.2 Experimental Methods:

3.2.1 Seeds weight:

Hundred seeds weight of the four varieties was carried out by weighing100 seeds samples by appropriate sampling procedure**.**

3.2.2 Kernels to hulls ratio:

Delinted cottonseed of the four varieties were cleaned from any impurities and undesired materials. Then hundred seeds from each type were taken and weighted. They were then dehulled and the weights of hulls and kernels were reported.

3.2.3 Moisture and volatile matter:

The moisture and volatile matter of seeds from the four varieties were determined on the ground seeds according to A.O.C.S official method Aa 3-38 revised 1952 and 1969, revised 1972 revised 1974.

This method determines the moistureand any material which is volatile under the conditions of the test.

$$\text{Moisture and volatile matter \%} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

3.2.4 Oil content:

The oil content of the seeds obtained from all the varieties under investigation were carried out according to A.O.C.S 4-38 revised 1972, A.O.C.S official method Ab 3-49 revised 1979.

$$\text{Oil in ground sample \%} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

The percentage oil can be calculated to any moisture basis with the following formula:

$$\text{Oil desired moisture basis\%} = \frac{\text{F (100 - \% moisture dried)}}{\text{100-moisture in ground sample}}$$

F = % Oil determined in ground sample .

There fore ,Oil % dry weight basis=

$$\frac{\text{(\%Oil in ground sample)} \times 100}{\text{100 - \% moisture in groundsample}}$$

3.2.5 Solvent extraction:

Oil was extracted by solvent extraction according to A.O.C.S. specifications H2-41. using n.hexane. Each variety sample of seed were extracted for oil without hexane as asolvent in large scale (kg sample). Soxhelt extractor to produce Large quantities of oil in the lab. The extraction usually lasted 8 hours. The extracted oil was detected, then cooled to room temperature and kept for further analysis and processing.

3.3 Gossypol Extraction

Gossypol extracted by petroleum ether and Malic acid, then gossypol was detected by a thin -layer chromatography (T.L.C).

3.3.1 Thin -layer chromatography investigation (T.L.C):

3.3.1.1 Preparation of the Layer:

A thin Layer of silica gel was spread on glass plate, the sorbent contained 5% of abider calcium sulfate to improve adhesion to the plate, The thickness of the plate was 0.02mm, after spreading the Layer was activated by drying in oven at 100° C for 30 minutes. After cooling the plate was eighther used directly or stored in a desiccator.

3.3.1.2 Methods of development and detection:

After the plates was activated, equall volume from four different oil sample (Barakat 90, Hamid, Ex_1) were spotted near the lower edge of the plate using a micro s– syring. The applied samples were allowed to dry at room temperature and the spotted plate was then placed in acovered glass tank in an up right position with the lower edge immersed in the solvent at bottom of the tank. The solvent system (benzene, petroleum ether, acetic acid 90:10:4) were used. When the solvent front traveled a dried distance the plates were then removed from the tank, adrid 5-10 minutes at room temperature. The spots were made visible by sprayed with iodine vapor.
Then the mobility parameter (R_f) for each spot was calculated according to the following equation:

$$(R_f) = \frac{\text{Distanced component moved}}{\text{Distance solvent moved}}$$

3.3.2 Determination of gossypol in seed cake and oil using high performance liquid chromatography(HPLC)

The HPLC procedure optimized by Marquié and Borrély (1991) was used to analyzed the gossypol in theseed cake and oil.After being –peeled, cut and weighed, the seeds were ground and sieved (30 mesh).

Sample of ground seed (100 mg each) (5ml litter in case of oil) were hydrolyzed for 10 min in boiling water bath at 100 °C with 20 ml of glacial acetic acid. At the same time two samples (1-2 mg) of standard gossypol (sigma ref –G-8761, St.Louie, MO) were treated similarly.

The solutions were filtered through silanized glass wool into 50-ml volumetric flasks. The residues were rinsed three times with 2 to 3ml of water \ acetonitrile (50:50;v\v) mixture, the recovered solutions were diluted up to 50 ml and homogenized carefully. The samples were then left at room temperature for 3h before being filtered through a 0.20- µm nylon membrane (MSI).

The samples were directly analyzed on a Merck Hitachi L 6200 chromatograph (Hitachi) Ltd., Tokyo, Japan) equipped with a Merck Hitachi L4000 UV. The chromatographic signals were integrated on a Hewlett Packard HP 1000 integrator (Hewlett Packard, USA). Other analytical conditions were fixed As follows (i) column- Inertsil 5 µm ODS-3 from chrompack (The Netherlands); (100×3mm); (ii) mobile phase-acetonitrile\water (acidified to pH =2.6 with phosphoric acid) 88:12 (v\v) at flow rate of 0.5 ml min^{-1}; (iii) UV detection at 272 nm; and (iv) duplicates of 20-µl injections were made for all samples. Standard curve for gossypol was constructed from triplicate determinations each for gossypol quantities of 0.5mg, 1mg, 1.5mg, and 2mg.

CHAPTER FOUR

RESULTS AND DISSCUSION

4.1 Weight of hundred seeds

Hundred seeds weight of the different cottonseed varieties are shown in Table (1). From the results Abdein and Barakat 90 showed the highest weight of 11.02 g and 10.70 g respectively.

Table (1): Weight of hundred seeds (g) of four cottonseed varieties:

Variety	100-seeds weight (g)
Abdein	11.02
Barakat 90	10.70
Hamid	10.44
Ex_1	09.26

4.2 Kernel to hulls ratio

Kernel Hulls ratio (wt/wt) of four cottonseed varieties are presented in Table (2). Barakat 90 showed the high kernel hulls ratio (1.92) followed by Hamid and Ex_1, which Abdein showed the lowest kernel hulls ratio (1.34).

Table (2): Kernel (wt%), hulls (wt%) and their ratio of four cottonseed varieties:

Variety	Kernel (wt%)	Hulls (wt%)	Kernel\Hullls(ratio)
Abdein	57.44	42.55	1.34
Barakat 90	65.79	34.20	1.92
Hamid	64.46	35.53	1.81
Ex_1	58.96	41.03	1.43

4.3 Moisture and volatile matter for whole seeds:

The moisture content of the four cottonseed varieties are shown in Table (3). The moisture content of the seeds for all varieties was almost the same.

Table (3): Moisture and volatile matter (%) for whole seeds of four cottonseed varieties:

Variety	Moisture and volatile matter%
Abdein	5.25
Barakat 90	5.17
Hamid	5.10
Ex_1	5.28

4.4 Oil content:

The oil content of the different cottonseed varieties are shown Table (4) shows. Hamid and Barakat 90 gave 21.12%, 20.78% respectively**.**

Table (4): Oil content (%) in whole seed of four cottonseed varieties:

Variety	Whole seed%
Abdein	19.77
Barakat 90	20.78
Hamid	21.12
Ex_1	19.39

4.5 Thin-layer chromatography investigation:

The mobility parameter (R_f) values for the best developing solvent system were recorded in table (5).Five compound were isolated from four samples of crude extract of seed cake, compound number 2 (Rf=0.6) compound number 5 (Rf=0.9) are the same inthe colour and in the distance.

Table (5): (R_f) values for TLC solvent system of four Cottonseed Oil varieties

Components	Sample				
	Barakat 90	**Ex_1**	**Abdein**	**Hamid**	**Band Colors**
Rf_1	0.5	0.2	0.2	0.2	Brown
Rf_2	0.6	0.6	0.6	0.6	Yellowish orange
Rf_3	0. 7	0.7	0.7	0.6	Different coulours
Rf_4	0.8	0.8	0.7	0.7	Dark brown
Rf_5	0.9	0.9	0.9	0.9	Yellowish orange

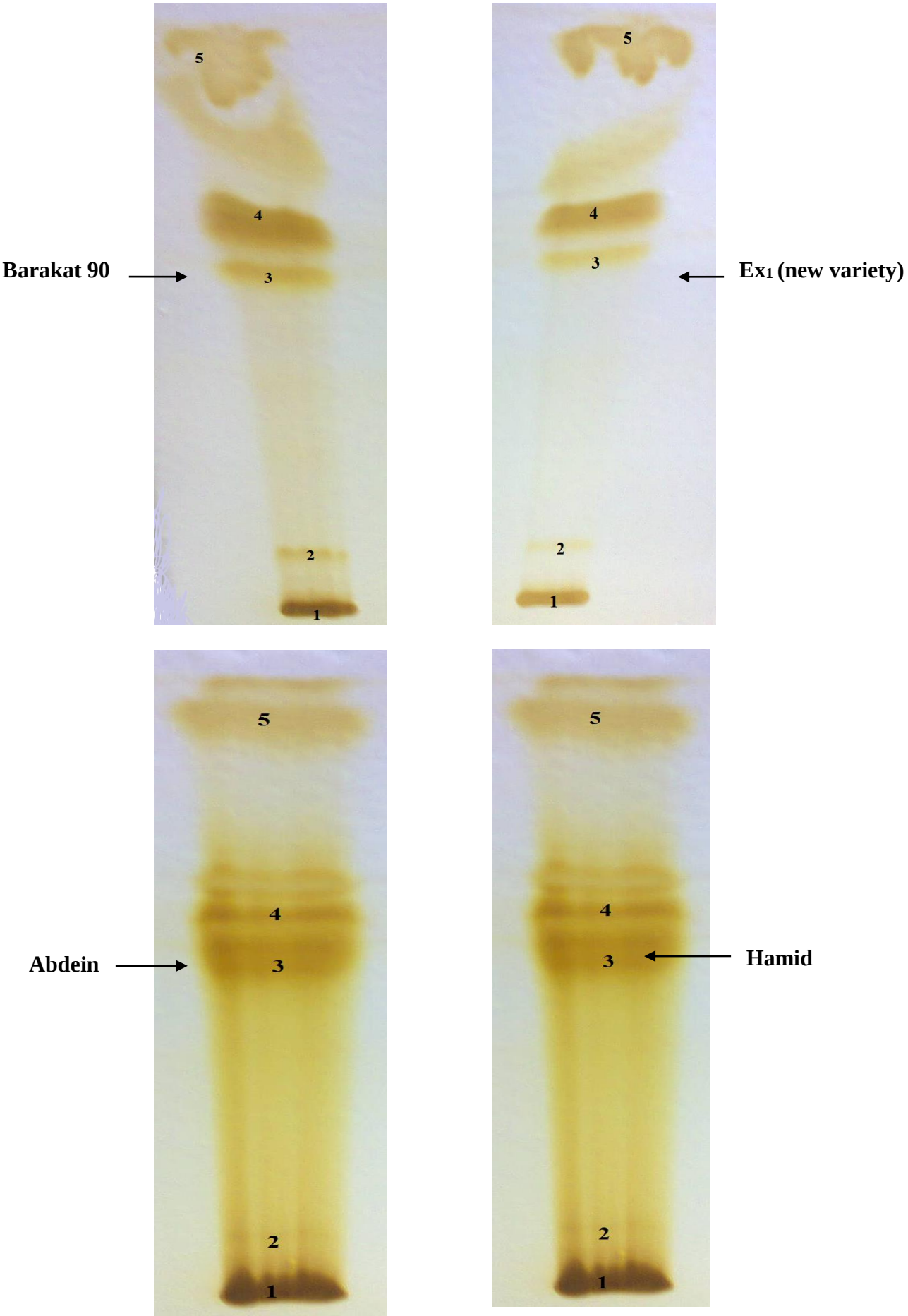


Fig3: A photograph of TLC separated components of the cottonseed oil

4.6 Determination of gossypol in seed cake using high performance liquid chromatography (HPLC):

Gossypol content in cake of four cottonseed varieties are presented in table (6). Barakat 90 gave The high amount of gossypol in cake (1.20%) while Hamid gave the low amount of gossypol in cake (0.31%).

Table (6) gossypol content (%) in seed cake of four cotton seeds cultivars:

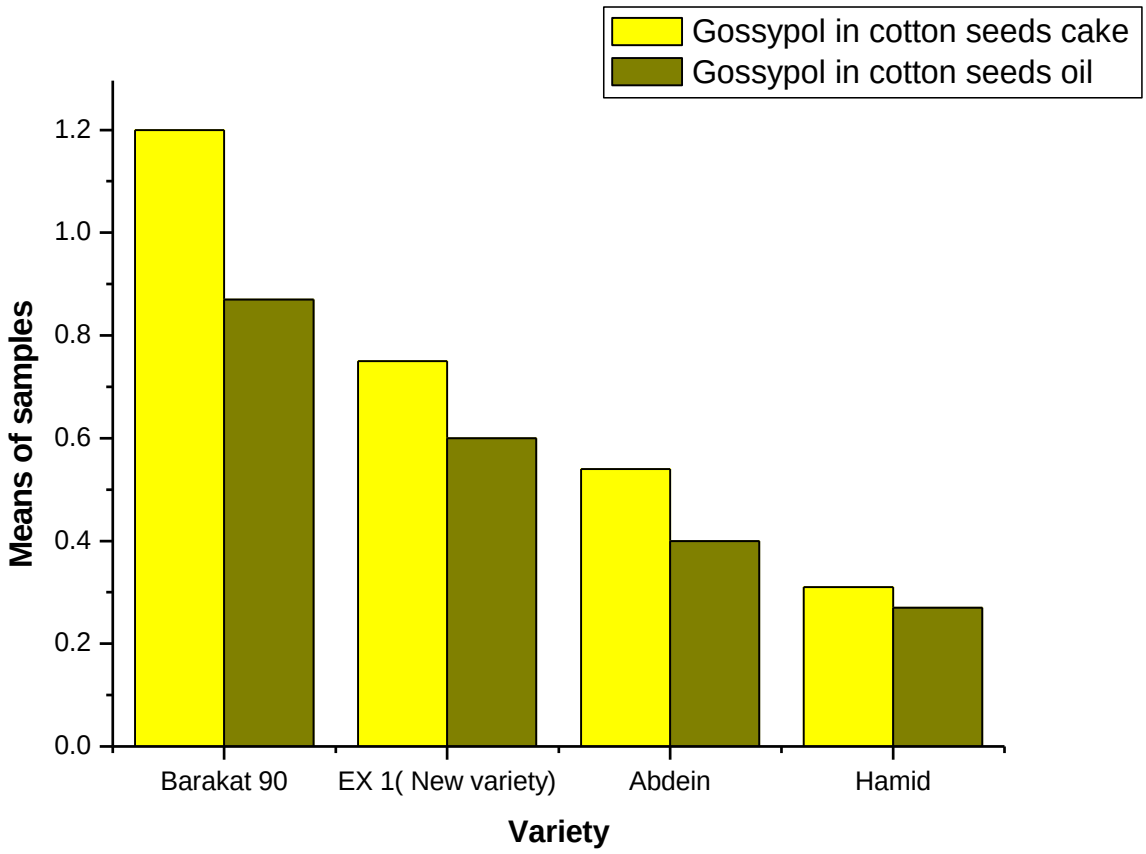
Variety	No. of Samples	Range	Mean	Std.Dev	Std.E
Barakat90	3	1.18-1.22	1.20	0.20	0.11
Ex_1	3	0.73-0.76	0.75	0.17	0.10
Abdein	3	0.52-0.56	0.54	0.02	0.01
Hamid	3	0.29-0.33	0.31	0.02	0.01
Total	12	0.29-1.22	0.70	0.34	0.09

4.7 Determination of gossypol in oil using high performance liquid chromatography(HPLC):

Gossypol content in oil of four cottonseed varieties are presented in table (7). Barakat 90 gave The high amount of gossypol in oil (0.87%) while Hamid gave the low amount of gossypol in oil (0.27 %).

Table (7) gossypol content (%) in oil of four cotton seeds cultivars:

Variety	No. of Samples	Range	Mean	Std.Dev	Std.E
Barakat90	3	0.85-0.89	0.87	0.020	0.115
Ex_1	3	0.58-0.62	0.60	0.020	0.115
Abdein	3	0.38-0.42	0.40	0.020	0.155
Hamid	3	0.26-0.29	0.27	0.015	0.008
Total	12	0.26-0.89	0.53	0.235	0.068



Fig(5): Gossypol in cottonseed cake and oil

4.8 Discussion:

In this study gossypol was extracted from seed and oil of three cotton cultivars which are currently grown in the Sudan and an experimental line (Ex_1). Different extracting methods of gossypol were used. The amount of gossypol in seed cake and oils were determined.

Weight of hundred seeds: Hundred seeds weight of cottonseed varieties was determined (Table 1), 100 seeds weight of Abdein was greater than barakat 90, Hamid and Ex_1 respectively, the difference may be due to genetical background in addition to the effect of environment climate and season.

Hulls: Kernel ratio: Hulls:Kernel ratio for delinted cottonseed were determined, Barakat 90 and Hamid has a high kernel weight between cultivars (Table 2), this indicate more oil yield in addition dehulling will increase oil recovery in pressing or solvent extraction (Norris, 1982).

Moisture and volatile matter: Hamid are less moisture content between varieties table (3). The moisture content of cottonseed reported by M.E Hameed is in the range between (4.47-4.69) (Hameed, 1998).

Oil content: Hamid has a high oil content with respect to the other three types, the results reported in table (4). The high oil content on Hamid and Barakat 90 between the varieties of oilseeds may be due to the healthy plants in the absence of cotton crop pests and diseases. This also yielded high quality oil without any pesticide residues. The oil content in the whole seed ranged between (19.39- 21.12). The oil content in Barakat 90 (20.78%) this result not agree with M.E. Hameed which reported that Barakat 90 has a high oil in whole seed is 24.5% than other this difference in oil content may be due to some straine durig 2012 at which the samples were collected.

Mobility parameter (Rf) values for TLC solvent system: In the four sample of crude extract of seed cake, five compound were isolated rich sample (table 5), compound (number 2Rf = 0.6 compound number 5 Rf =0.9) are the same in all, in color and in distance so it may be the same compound in each (gossypol or gossypol derivatives) .

Gossypol content (%) in cake of four cottonseed cultivars: The high amount of gossypol extracted was from cake of all cottonseed cultivars in Barakat 90 (1.20%) compared to other cultivars, slightly low in Ex_1 (0.76%) and further low in Abdein and Hamid cultivars (0.54%) and (0.31%) respectively (table 6), This result agree with the Setelo (2005) confirmed that gossypol concentration varied among the species also among varieties of the some species. M.E Abdo (2012) reported that Barakat 90 has a high amount of gossypol in cake between other cultivars (0.90%).

Gossypol content (%) in oil of four cottonseed cultivars: The high amount of gossypol extaracted was from oil of all cotton cultivars in Barakat 90 (0.87) % compared to other cultivars then Ex_1 and Abdien respectively and further low in Hamid (0.27%) (table 8). The amount of gossypol in oil of Barakat 90 and Ex_1 are high than the save level 0.45% which reported by the Food and Drug administration USAFDA (Cherry, 1983).

In this study we found that there is relationship between the amount of gossypol in seed cake and oil (Cultivars which carried high amount of gossypol in seed cake also carried high amount in oil).

Also it was observed that the amount of gossypol was less in oil of each cultivar than in seed cake of these cultivars.

Different brands of edible oils and ghee are used for cooking and frying of food and also used in sweet, bakery product etc. If gossypol is

accumulated in human body it may cause harmful effects (USDA/Agricultural Research service, 2007).

(Hron *et al* .,1987) reported that some amount of gossypol tends to react with many natural substances in cottonseed and forms the bound gossypol that is non-harmful. However the unreacted gossypol known as "free gossypol" is toxic. Thus free gossypol is an anti-nutritional factor that limits the use of cottonseed and its products (Hron *et al*., 1987).

The toxicity of gossypol is associated to the reaction of its phenolic groups to amino acids and minerals. Hydrogen bonding and oxidation of the carbonyl groups result in easily reactive quinones that bind with proteins. Heating of cottonseeds during oil extraction binds gossypol to proteins. Thus reduces protein availability from cottonseed meal (Sharma and kumar, 1999).

A large- scale contamination of cotton oil for human consumption with gossypol cause infertility incident (Qian and Wang 1984; Amini and Kamkar, 2005).

(Wu, 1989) reported that home made unheated cottonseed oil developed fatigue and a burning sensation on the face and other exposed parts of the body and these symptoms were qualified as "Hanchuan fever or burning fever. However, Gossypol has also been shown to have in Vitro and in Vivo inhibitory diverse pathogenic agent such as *Trypanosoma cruzi* (Montama *et al* 1982; Abe *et al*; 2004). Its used in medicine because of its anti –tumor and anti –carcinogen properties and provides endurance against plant mites. Fungus and bacteria. Its also antifertility properties in male (Cass *et al*., 1991; Vroh Bi *et al*., 1999; Benson *et al*., 2001; Coutinho, 2002; Qiu *et al*., 2002; Cai *et al*., 2004; Meyer *et al*., 2004; Sotelo *et al*., 2005; Kline *et al*., 2008; Moon *et al*., 2008). Gossypol inhibits growth and development of many insects (Bottger and Pottana, 1966).

During this study, the gossypol extracted was higher in seeds of Barakat 90 compared to other cultivars. The amount and/or concentration of gossypol in plant tissues depends on environmental factor (Cherry *et al.*, 1978), on the species of the cotton (Boatner *et al.*, 1949) and on gland density (Lukefahr *et al*.,1966). Some workers reported that several toxic substances related to gossypol were found in some cotton parts. Example of such substances are p-hemigossypolone, p-hemigossypolone-6-methyl ether, heliocide H_1, heliocide H_2, heliocide H_3, heliocide H_4, heliocide B_1, heliocide B_2, heliocide $_3$ and heliocide B_4 (Gray *et al.*, 1976; Stipanovic *et al*., 1977). These substances have high activity against cotton pests (Hedin *et al*., 1992). Gossypol significantly drew attention because of its toxicity and also for being localized in seeds having nutritional value i.e higher oil and protein contents (Moore and Rollin, 1974) .

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATONS

5.1 Conclusion:

1. Hamid cotton variety has a higher oil content in whole seed between the other three cultivars and the lower amount of gossypol concentration in both seed cake and oil between the different other cultivars.

2. The amount of gossypol extracted from seed cake and oil by high performance liquid chromatography method varied with cultivars i.e being higher in Barakat 90, Ex_1, Abdein And Hamid .

3. Barkat 90 cotton variety has a highest gossypol content in both seed cake and oil .

4. The amount of gossypol concentration in oil of Barakat 90 andEx_1 varieties are a highest (0.87, 0.60 % respectively) than that of the safe level reported by the Food and Drug administration of the USAFDA (0.45%) (Cherry,1983).

5.2 RECOMMENDATONS:

1. This study recommend that Hamid and Abdein variety is much recommended to be used for oil production for its low gossypol content, as Barakat 90 should be treated and refined or extracted with optimum method to raise the oil content and to reduce gossypol.

2. Further study should be done on gossypol like environmental factors that affect on gossypol content .

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