SEED OIL IMPROVEMENT IN COTTON

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Cotton, the single largest natural source of fibre worldwide and one of the most important commercial crops of India, plays a dominant role in both agrarian and industrial economies. In India it is the backbone of textile industry, which consumes 70% of the country's total fibre produced, accounts for a sizeable chunk in the country's export and fetches over a huge sum annually to the exchequer. But despite these rich tributes this all is just a part of the cotton story. The fact still remains that it produces twice as much seed as it does produce fibres. Among various forms of cottonseed utilisation, cottonseed processing for oil holds a prominent position.

1.0 Cottonseed Oil

Cottonseed contains hull and kernel. The hull produces fibre and linters. The kernel contains oil, protein, carbohydrate and other constituent such as vitamins, minerals, lecithin, sterols etc. Cottonseed oil is extracted from cottonseed kernel. With three times as much unsaturated as saturated fatty acid, cottonseed oil is considered by health organisations to be a healthful vegetable oil acceptable for modern diets. Cottonseed oil does not have to be as fully hydrogenated for many purposes as some of the more polyunsaturated oils. Refined and deodorized Cottonseed oil is one of the purest food products available. Processed cottonseed oil is the fifth leading vegetable oil in the world and the seeds are a vital source of protein and calories in the cattle and dairy industries.

1.1 Cottonseed Oil Composition

Fats and oils are composed mainly of triglycerides, three molecules of fatty acids joined to a glycerol molecule. The chain length of the fatty acids and their organisation on the glycerol backbone vary greatly. Fats and oils are a combination of fatty acids, both saturated (C14:0, 16:0, etc.) and unsaturated (C 18:1, 18:2, 18:3). Cottonseed oil also termed as "Heart Oil" is among the most unsaturated edible oils. It has a 2:1 ratio of polyunsaturated to saturated fatty acids and generally consists of 73% unsaturated fatty acids including 18% monounsaturated (oleic) and 55% polyunsaturated (linoleic) and 27% saturated (palmitic and stearic) fatty acids (Table 1). Cottonseed oil is cholesterol free, as are all oils extracted from plants. An additional benefit from Cottonseed Oil is the high level of antioxidants - tocopherols. Studies show that these natural antioxidants are retained at high levels in fried products, preserving their freshness and creating longer shelf life.

Table 1 :Typical Fatty Acid Composition in different forms of Cottonseed oil

FATTY ACID	COTTONSEED COOKING OIL	COTTONSEED SALAD OIL	PARTIALLY HYDROGENATED
Myristic (14:0)	0.8	0.8	0.9
Palmitic (16:0)	24.4	22.3	22.5
Palmitoleic (16:1)	0.4	0.4	0
Stearic (18:0)	2.2	2.0	5.5
Oleic (18: 1)	17.2	16.7	50.0
**Linoleic (18:2)	55.0	57.6	20.3
**Linolenic (18:3)	0.3	0.3	0.3
SUMMARY			
% Saturates	27	25	29
% Monounsaturated	18	17	50
% Polyunsaturated	55	58	21

Cottonseed oil is generally clear with a light golden colour, but like most oils, the degree of colour depends on the amount of refining. Clear, colourless oils are not necessarily better oils, but may have been refined more severely. Cottonseed oil's light, non-oily consistency and high smoke point make it most desirable for cooking. Analytical values on different parameters for different cottonseed oil forms are given in Table 2.

Table 2 : Typical analytical values for cottonseed oil products

	Cottonseed Cooking Oil (RBD)*	Hydrogenated Cottonseed Shortening
Lovibond Colour (Red Max.)	2.0-6.0	2.0-2.5
Free Fatty Acid (as Oleic % Max.)	0.05	0.05
Peroxide Value (Meq/kg. Max.)	1.0	0.5
Iodine Value	103-116	50-70
AOM Stability (hrs.)	15	100-200+
Cloud Point (°F)	30-38	-
Melting Point (°F)	50-60	100-118
Pour Point (°F)	-	102-140
Smoke Point (°F)	430	-
Cold Test (hrs.)	-	-
Flavour	Bland	Bland
Density (lb/gal @ 108°C)	-	7.46

* RBD - Refined, Bleached & Deodorized

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1.2 Cottonseed Processing

Although cotton cultivation dates back to prehistoric times, cottonseed processing is of recent origin. Cottonseed processing industry had its beginnings with the invention of the cotton gin in 1793 by Eli Whitney, which made the availability of large supplies of cottonseed possible. However, it was in the early twentieth century only that cottonseed was utilised in commercial quantities for production of oil and decorticated cottonseed cake. In India traditionally, the cottonseed utilisation has been as an animal feed and it was in 1914 that Indian Oil Company established the first cottonseed oil mill at Navsari. Since then the Indian cottonseed oil industry has come a long way. Today, the value of cottonseed represents about 18% of a cotton producer's income. In India cottonseed production and availability in recent years is given in Table 3.

Table 3 : Estimated Cottonseed Production and Availability for Processing

	2006- 2007	2005-2006
Lakh Bales of Cotton (170 Kg. each)	270.0	250.0
Cottonseed Production (Lakh tons) @333 Kg/Bale	90.0	85.0
Retained for Sowing & Direct Consumption (Lakh tons)	11.5	11.5
Marketable Surplus (Lakh tons)	78.5	73.5
Production of Washed Cottonseed Oil (11%) (Lakh tons)	8.63	8.0

1.2.1 Scientific *vis a vis* Traditional Processing

Cottonseed could either be processed by the traditional crude method of crushing seed without delinting in undecorticated form or by scientific processing of cottonseed, which involves removal of linters, decortication, separation of hull, expelling, solvent extraction and refining of oil. Figure 1 illustrates the entire process of scientific cottonseed crushing. There has been a gradual improvement in quantity of cottonseed processed scientifically in the country in the last three years.

Nearly 1,93,396 tonnes of cottonseed was processed scientifically during the year 2001-02 as compared to 1,53,484 tonnes in 2000-01 and 1,11,414 in 1999-2000 (Bajoria, 2002). But considering the potential this improvement is still the tip of an iceberg. Scientific processing results in extraction of entire cottonseed oil, while the oil cake obtained by traditional method still contains about 7% residual oil. This high residual oil is not required by the animals but is a misconception among the dairymen. The cottonseed meal obtained through scientific method contains negligible oil and has very high by-pass type protein content of 40 to 42%. In addition scientific processing results in a number of useful by-products such as linters, hulls and cottonseed extract.

Figure 1 : Scientific processing of cottonseed oil

Cottonseeds
↓
Delinting
↓
Decortication
↓
Separation of Hulls
↓
Expelling
↓
Solvent Extraction

1.3 Cottonseed Oil Usage

Cottonseed Cooking Oil has a bland, neutral flavour that does not mask food flavours. It is ideal for frying and fine for cooking, salad dressings and other applications. Cottonseed oil is primarily used in the U.S. as a salad or cooking oil. About 56% is consumed in that category while about 36% goes into baking and frying fats, and a small amount into margarine and other uses. In India nearly entire cottonseed oil produced is utilised for edible purposes, mostly for *vanaspati*, only small quantity (5-10%) is used for manufacturing soaps. Cottonseed oil can be used in all kinds of culinary applications and can be customised to fit into a number of unconventional usage (Table 4).

Table 4 : Cottonseed oil and food manufacturing applications

Use	Remark
Deep frying	Cottonseed oil is an excellent flavour carrier and enhances rather than masks the fresh, natural flavours of foods.
Baking	Blending of fully hydrogenated cottonseed oil with a partially hydrogenated base stock creates a shortening with a solid fat index that achieves an optimum plasticity for baked goods.
Margarines, icings and whipped toppings	Cottonseed oil is noted for its ability to form the beta prime crystal, which helps promote the desired consistent texture and smooth, creamy appearance in shortenings, toppings and spreads.
Salad dressings	Excellent choice for salad dressings owing to its neutral flavour profile and the fact that it can be winterised- a necessity for mayonnaise and commercial flavoured dressings. Dressings made with cottonseed oil resist oxidation well.

1.4 Transgenics and Cottonseed Oil

Since in March 2002 Govt. of India has approved commercial production of *Bt* cotton in India, it is but natural to be concerned about health hazard related to consumption of biotechnology derived products. In USA where Cottonseed Oil ranks third in terms of volumes among edible oils, the US National Products association has stated that oil refined from genetically engineered cotton seed varieties does not carry any risk for consumers (ICAC Recorder March, 2001). It further stated that refined Cottonseed Oil could be safely consumed because no protein content resulting from DNA, native or otherwise was detected in the oil.

Further in an evaluation at CICR, no significant differences were observed for seed oil content between *Bt* hybrids and their non *Bt* counterparts. In a yet another study also conducted at CICR, ELISA procedure was employed for detection of *Cry1 Ac* protein present in crude and refined oil obtained from *Bt* cotton. Refined oil samples of *Bt* cotton did not show the presence of *Cry1 Ac* while the crude seed oil samples of *Bt* cotton showed about 100 pg *Cry1 Ac* protein in 500 µl oil. The presence of *Cry1 Ac* only in crude oil samples indicated that the protein is not present in the oil but is present in the seed debris that is usually present in the crude extractions.

2.0 Genetic Improvement of Cottonseed Oil

In cotton there are four cultivated species, two tetraploid species namely American cotton *G. hirsutum* and Egyptian cotton *G. barbadense* and two diploid desi cottons *G. arboreum* and *G. herbaceum* . The seeds of desi varieties are small in size in comparison to tetraploid species and contain lower percentage of seed oil. Desi and American seeds normally have significant amount of linters whereas Egyptian cotton seeds are without linters.

The range of oil content and fatty acid content of four cotton

Name of the species	Oil %	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid
G. arboreum	16.6-22.1	23.1-25.9	2.3-3.4	20.8-6.3	41.1-50.6
G. herbaceum	16.9-22.4	20.5-23.4	3.2-4.4	17.5-20.8	51.3-55.1
G. hirsutum	16.1-24.4	23.1-28.0	2.4-3.4	14.7-20.9	47.6-55.4
G. barbadense	17.9-25.6	24.4-25.5	2.6-3.0	18.7-19.7	50.0-51.7

2.1 Genetic Variability for Cottonseed Oil Content and Quality

Various workers world-wide have reported a wide array of variability for seed oil content in cottonseed. This could be an interesting and useful phenomenon but for a bottleneck that a sizeable proportion of this variability had its reasons beyond *genetics*. Therefore, it would be important to quantify the genetic variability for cottonseed oil content and quality in order to set the realistic achievable goals. In Indian context various researchers have reported a broad range of variability.

The extent of variability has been reported to be higher in tetraploid cotton than in diploid. The extent of variability observed in germplasm of four cultivated species indicated ample scope for genetical improvement of cottonseed oil through hybridisation followed by directional selection. Similarly for oil quality a broad range of variability for different fatty acid profiles in cultivated cotton species in India has also been studied by various workers (Table 5).

Table 5. Extent of Variability for Seed Oil % in Cotton

Extent of variability witnessed in fatty acid profiles in above mentioned studies for all the cultivated cotton species could well be utilised in developing lines with high polyunsaturated and monounsaturated fatty acid contents through appropriate breeding techniques.

Species	Genotypes	Extent of	Reference
G. arboreum	337	12.5 - 22.9	Singh and Singh,
G. herbaceum	96	13.5 – 20.4	Singh and Singh,
G. arboreum	Nearly	14.4 – 24.5	Singh, 1988
G. barbadense	162	14.0 – 25.8	Singh, 1988
G. hirsutum	914	12.9 – 29.8	CICR, 1989
G. barbadense	242	14.0 – 25.8	CICR, 1989
G. arboreum	149	15.1 – 23.4	CICR, 1989
G. herbaceum	346	13.5 – 20.4	CICR, 1989
G. hirsutum	176	10.2 – 26.1	Dani et al., 1997
G. arboreum	79	14.8 – 25.8	Dani et al., 1997
G. hirsutum	-	16.1 – 24.4	Pande, 1998
G. barbadense	-	17.9 – 25.6	Pande, 1998
G. arboreum	-	14.6 – 22.1	Pande, 1998
G. herbaceum	-	16.9 – 22.4	Pande, 1998

2.2 Breeding Objectives and Progress made

Breeding objectives for cotton traditionally have been to improve fibre productivity & quality and protection from stress, both biotic and abiotic. The processing of cottonseed for oil has howsoever received little attention from an applied cotton improvement perspective. Thus assessing the potential for improving the cotton cultivars for high oil productivity and quality and keeping in mind the future consumption, research work was initiated in the last decade at Central Institute for Cotton Research (CICR), Nagpur in collaboration with USDA, USA. Earlier work was aimed at preparing a data base on cottonseed oil content and quality of widely cultivated *G. hirsutum* and *G. arboreum* varieties/ land races and germplasm lines including material received from U.S.A. A number of lines identified for high oil content and quality have been used in later breeding programmes that are mainly focused on the following aspects:

i. Reduction in the portion of hull and linters in cotton seed and increase the kernel.
ii. Increase in cottonseed oil and protein content.
iii. Genetic enhancement of polyunsaturated fatty acid profile.
iv. Decrease or elimination of *Gossypol* content.

Introgression of high seed oil content into early maturing agronomic bases have resulted in identification of a few high yielding cultures which mature in 135-140 days and are under multilocation testing (Mayee and Waghmare, 2001). An early maturing high yielding line "CNO 131" has already been registered with NBPGR, New Delhi (Dani, 2000). More recently, to register a speedy progress on seed oil improvement front, a number of lines have been identified as potential donors (Table 6) at CICR in Technology Mission on Cotton, Mini Mission I, under the aegis of Government of India, for utilisation in creating further selection base as well as in conversion programme to combine high seed oil with fibre productivity and quality.

Table 6 : Potential donors for higher oil content

Hirsutum Germplasm Lines	Oil %	Arboreum Germplasm Lines	Oil %
DL 1	26.21	A 14	24.5
GITHV 4190	25.47	LD 134	24.4
CISV 17	25.06	V 434	24.3
CIR 56 P1	25.01	H 168	23.9
CISV 60	24.98	H 336	23.9
CISV 52	24.86	H 94	23.9
KH 2	24.47	H 171	23.8
CRB 13 P3	24.47	N 1123- NR	23.5
RS 810	24.14	LD 191	23.4
CRB 21	24.08	Gao-CB-4	23.2

As an outcome of a decade long breeding programme at CICR and also the research carried out under TMC umbrella a number of advanced stabilized cultures possessing high oil content and productivity have been identified which would undergo rigorous testing under multilocation trials in ensuing years.

The availability of successful commercial hybrids in cotton offers prospects in their exploitation for seed oil, however, research work conducted so far on heterosis for oil content suggests only marginal hybrid vigour for the trait but still because of hybrids' higher productivity potential they will eventually always give higher seed oil yield per hectare.

2.3 Biotechnology for Oil Improvement

Developments in gene manipulation techniques have provided new tools for generating novel plant phenotypic expressions otherwise not possible through conventional plant breeding techniques. Herbicide tolerant and insect resistant *transgenics* have already made long strides in terms of achievements in several crops including cotton. Cotton being an oil-yielding crop also offers tremendous opportunities where novel genetic engineering techniques could chip in with valuable contributions.

2.3.1 Marker Aided Selection (MAS)

The advent of new molecular markers such as RFLP, AFLP and RAPD has demonstrated that these could be used as a powerful selection tool for enhancing selection efficiency and curtailing time and resources involved in traditional selection procedures In cotton using such markers, some possibilities have been expressed, aiming towards improvement of quantitative traits (Meredith, 1994).

2.3.2 Making Cottonseed Oil Healthier

Cottonseed oil is extensively used in food industry for several purposes but it is generally subjected to hydrogenation, which renders it stable during cooking at high temperature and, also softens and provide *mouth feel* in baked products. But hydrogenation leads to production of *trans* fatty acids also in some amount, which are health hazards and raise the cholesterol level in the blood. To produce naturally hydrogenated cottonseed oil free from *trans* fatty acids, scientists at Plant Industry Division, CSIRO, Australia have reintroduced a small amount of cotton's own DNA into its genome to *switch off* the gene which converts monounsaturates to polyunsaturates (ICAC Recorder, 2001) (Figure 2). This results in cottonseed oil with high level of polyunsaturated fatty acid and, hence, avoiding any need for hydrogenation.

2.3.3 Improving functional property

In a related development, the CSIRO research team has also successfully used gene technology to alter the proportions of saturated fatty acids in cottonseed oil. Saturated fatty acids provide the solid properties that make cottonseed oil useful in margarine production. About a quarter of cottonseed oil is made up of two saturated fatty acids, called palmitate and stearate. Conventional cottonseed contains mostly palmitate, with small amounts of stearate. Nutritionists believe that stearate does not raise blood cholesterol, but palmitate does. The team of researchers has modified the cottonseed so that it produces stearate instead of palmitate, making it a healthier product for margarine. The first field trials of this *trangenic* would have commenced in 2002. If it progresses well, commercial varieties could be available to growers by 2004 with the first commercial harvest in 2005.

2.3.4 Gossypol free Cotton

The annual world-wide cottonseed yield could supply the dietary protein needs of some 240 - 350 million people, but presence of *Gossypol* is a major deterrent. Ruminant animals could tolerate the *Gossypol*, but it is toxic to non-ruminants. If Gossypol were not present, cottonseed oil could be made more economically and cottonseed meal could be processed for food and feed. The use of glandless cotton could produce *Gossypol* free cottonseed, but then insect predation would be a big menace. To keep *Gossypol* in plant but away from seed Ow (2000) proposed engineering the seed specific breakdown of *Gossypol*. He reasoned that *Gossypol* in cottonseed, like all organic matter must get recycled into the basic building blocks and that would mean there should be microbial enzymes that breakdown this compound. He identified an enzyme that could degrade the *Gossypol*, which ultimately led to the gene and construct for expression in plants. His group has been able to produce *transgenics* in which size and density of *Gossypol* containing glands were reduced in leaves and they have to further concentrate their work on getting the gene expressed in seeds, which would be the ultimate goal.

In yet another approach to reduce gossypol from the cottonseed, researchers are utilising antisense version of delta-cadinene synthase gene (Smith and Rathore, 2001), also driven by two different seed-specific promoters (cotton alpha-globulin promoter and French bean phaseolin promoter). The alpha-globulin promoter isolated has also been evaluated to ensure that it will drive various genes in a seed-specific manner. One of the important has been that the cotton alpha-globulin promoter (AGP), which is utilised for antisense work, has been functionally characterised. AGP can drive seed-specific expression of transgenes in several dicots (*Arabidopsis*, tobacco, and cotton), however, it is strongest in cotton. In gossypol reduction project, seeds from 111 plants were obtained and analysed for gossypol content and 13 lines were identified with seed gossypol content of less than half the level found in normal plants. These positive results on gossypol reduction were very promising and suggested that the strategy used was effective. Both fatty acid improvement and gossypol reduction traits will be of enormous value to cotton industry by making cottonseed oil more competitive to other oils and by improving the value and nutritional quality of the meal.

3.0 Cottonseed Oil Processing and Keeping quality - Improvement

To address problems associated with oil extraction and long term keeping quality research work, under TMC, is in progress at Central Institute for Research on Cotton Technology (CIRCOT), Mumbai to develop efficient oil extraction protocols and also to test their techno-economic feasibility. Efforts are being made to identify suitable antifoaming agent along with its optimum concentration for preserving quality of frying oil for longer duration under Indian cooking habits. To create awareness and to promote widespread consumption, work is being carried out towards exploring novel culinary applications also.

4.0 Potential for Cottonseed Oil Improvement & its Processing in India

1. There is a gap of 5-6% between the potential and realised oil content in current cultivars that could be bridged through intensive breeding efforts.
2. Development of high seed oil lines with glandless seeds and glanded vegetative parts would bring down the processing cost.
3. Further improvement in oleic acid content in cultivars may circumvent the need for hydrogenation.
4. Introduction of *Bt* gene into glandless types would be remunerative in terms of avoiding processing cost and insect predation.
5. The modern breeding techniques such as *transgenic* development, molecular breeding and marker aided selection may be rewarding in developing lines with high seed oil content and quality.
6. Modernisation of machinery for delinting and dehulling would lower the processing cost and make the scientific processing competitive in terms of net return.

5.0 Constraints for Cottonseed Oil Improvement, Processing and Utilisation

1. Fibre or lint yield in cotton is determined by two major factors: the number of seeds produced per acre and the weight of fibre produced on the seed. Cotton plants spend about 2.25 percent, as much energy to produce a pound of oil as the amount of energy needed to produce a pound of cellulose (Lewis, 2001). Ironically, if the weight of lint per seed is reduced (as has been the case since 1975 in U.S.A.), cotton plants must produce more seed per acre to yield the same weight of lint per acre. This places an extremely high demand for energy production on the plants and makes them much more susceptible to stress. Perhaps this is the reason yields have become stagnant and less reliable.
2. Processing technology and machinery used in scientific expelling of cottonseed oil is old and results in high processing cost an important impediment towards adoption of scientific processing.
3. Extension agencies have not been effective so far in dissipating misconception about cottonseed extract among dairy industry and also to create awareness among masses regarding cottonseed oil's benefits.
4. An important part lacking on oilseed industry's part has been the absence of conscious efforts towards branding. The absence of established oil majors and effective tele advertising has been the reason for the apathy of general public towards cottonseed oil.

Suggested Further Readings

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